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Wheat breeding for breadmaking quality has exploited most of the existing genetic variation in the High-Molecular-Weight glutenin loci (*Glu-1A*, *Glu-1B* and *Glu-1D*). This investigation was undertaken to examine the contribution of the Low-Molecular-Weight (LMW) glutenin allelic variation (*Glu-3A*, *Glu-3B* and *Glu-3D*) to breadmaking quality, as determined by the SDS-Sedimentation Test, Alveograph Dough Strength (W), Tenacity (P) and Extensibility (L). The effects of protein content, hardness, and wheat-rye translocations were also studied. Selected spring wheat populations were used in three separate studies. Two collections of cultivars and advanced lines developed in Southern Brazil were grown for two years at East Farm near Corvallis, Oregon. Large differences between LMW alleles at *Glu-3A* and *Glu-3B* for dough strength were found, while LMW *Glu-3D* did not exhibit much polymorphism. The most favorable LMW alleles were in low frequencies in these populations. Hard wheats were found to give higher dough strength values than

soft wheats and a wheat-rye translocation (1BL.1RS) was found to adversely affect quality. Two recombinant inbred populations were developed from two crosses between lines with different LMW glutenin alleles. This provided the opportunity to avoid some confounding factors by making comparisons in more uniform genetic backgrounds. The differences in SDS-Sedimentation between segregating alleles at HMW and LMW loci confirmed the quality rankings observed in the former study. To further examine the impact of wheat-rye translocations on SDS-Sedimentation and Alveograph dough strength, tenacity, and extensibility, three cultivars and near-isogenic lines (1BL.1RS or 1AL.1RS) were compared for two years. Wheat-rye translocations reduced SDS-Sedimentation value, dough strength, extensibility and increased tenacity. LMW glutenin alleles seem to offer the potential to increase breadmaking quality by developing wheats with higher dough strength or by compensating for the negative effects of wheat-rye translocations.

EFFECT OF THE SIX GLUTENIN LOCI ON SELECTED BREAD QUALITY TRAITS IN WHEAT

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IN DEDICATION

to Luciana, my wife

and to

Gilda and Ottoni, my parents

Effect of the Six Glutenin Loci on Selected Bread Quality Traits in Wheat

1. Introduction

Breadmaking quality of wheat has been subject of research for more than a century. Even with advances in cereal chemistry, breeders have relied on physical properties of dough to select parents for crossing. With the advent of electrophoretic techniques, High-Molecular-Weight glutenins were studied and shown to affect breadmaking quality. Breeders widely used HMW glutenin allelic composition to choose parents and select progeny. Favorable HMW glutenin alleles can be fixed after a few generations, hindering further improvements in dough quality. A potential solution for the problem is to determine the contribution of the Low-Molecular-Weight (LMW) glutenin alleles to quality. This opportunity is now feasible with the development of a new glutenin extraction procedure (Singh *et al.*, 1991). The study of the six multiallelic glutenin loci can provide a comprehensive understanding about the genetics of breadmaking quality, as these loci code for the polypeptides involved in the gluten polymer, a key factor in determining dough quality.

The main objective of this investigation was to evaluate the contribution of the HMW and LMW glutenin alleles to the breadmaking quality of selected spring wheat populations. Other factors that influence quality were also taken

into account. Protein content (or percentage) was considered, as the viscoelastic properties of the wheat dough arise from the polymerization of proteins (glutenins). Dough strength should increase with protein percentage. Another important factor is the variation in kernel hardness. This trait affects milling and baking quality and market ability.

The impact of wheat-rye translocations on dough properties must also be evaluated. Such translocations are widely found in spring wheat germplasm and are agronomically beneficial. They affect storage protein composition. Commonly, LMW glutenins coded at *Glu-3B* are substituted by secalins, which are not able to polymerize protein and therefore do not contribute to dough strength.

Results of this investigation can provide for a more thorough understanding of the major factors that control breadmaking quality and their interrelationships. This would enhance the ability of the wheat breeder to develop cultivars with superior end use quality and to avoid a possible negative relationship between yield and protein percentage, i.e., preserve breadmaking quality with higher yields and lower protein content. It also can allow for the exploitation of the genetic variability for protein quality.

2. Literature Review

Due to its many end-use products, ease of storage and transportation and wide adaptation wheat continues to be one of the most important food crops. Today it is the first crop in tonnage, with approximately 550 million tons per year being produced. It is also the staple food for more than 35% of the world's population.

The main components of a wheat kernel are starch, proteins and lipids, with all three influencing the end use quality. *Quality* indicates "degree of goodness or worth" (Hornby, 1987). For wheat, it can be nutritional quality for food or feed, starch quality for noodles, or protein quality for bread, pastry, pasta, etc. The focus of this review is on breadmaking quality.

Wheat's unique breadmaking properties are due to its protein component, which can vary from 6 to 18 percent. Protein content is one of the most important determinants of wheat quality and market value. However, the simple determination of protein content is misleading in terms of breadmaking quality as there are large qualitative differences within protein *per se*. Wheat proteins were characterized by Osborne in the beginning of this century (Osborne, 1907). His classification was based on the sequential fractionation of several components based on their solubility. These include the water soluble albumin; the globulins, which are soluble in dilute salt solutions, those soluble in aqueous alcohol are

the gliadins, and the fraction soluble in dilute acid or alkali the glutenins. The Osborne classification is still in use today, although a reasonable overlap between the fractions does occur. The gliadins and the glutenins form the "gluten", the essence of wheat's breadmaking properties. Both gliadins and glutenins form intermolecular and/or intramolecular disulphide bonds and are responsible for the formation of the gluten polymer. Gluten can be easily obtained by removing starch, albumins and globulins, through washing with water. The residue is an elastic and viscous material, which can reach 85% of the total protein.

The glutenins were first studied with electrophoresis in the late 70's, when Payne *et al.* (1979) and Lawrence and Sheperd (1980) separated reduced glutenins into several subunits using SDS-PAGE electrophoresis. Later those subunits were divided into two groups: High Molecular Weight glutenins (HMW) and Low Molecular Weight glutenins (LMW), coded by *Glu-1A*, *Glu-1B*, *Glu-1D* (HMW) and *Glu-3A*, *Glu-3B*, *Glu-3D* (LMW), located at chromosomes 1AL, 1BL, 1DL (HMW), 1AS, 1BS and 1DS (LMW), respectively.

Based on amino acid sequence comparisons of the individual polypeptides, the gliadins and glutenins are now called prolamins, which were defined as "storage proteins that are deposited in developing endosperms of cereals. They are rich in glutamine and proline, and are insoluble in the native state in water or dilute solutions of salts" (Shewry *et al.*, 1990). The intracellular

insolubility is likely to favor the packaging of the proteins in the developing grains. Their amino acid content similarities is possibly due to the necessary solubility properties required for efficient storage (at grain filling) and subsequent use (at germination) (Shewry *et al.*, 1990). The unique viscoelastic properties of wheat dough are mostly due to a diversity of cross-linked subunits that form a large polymer. Therefore, the presence of residues that are able to form disulphide bridges (S-S) is a very important feature closely related to functionality.

2.1. The Wheat Prolamins

The wheat prolamins were recently divided into three major groups: the sulfur-poor prolamins (ω-gliadins), the sulfur-rich prolamins (α- and γ-type gliadins and LMW glutenins), and the High-Molecular-Weight prolamins (HMW glutenins) (Shewry *et al*, 1986).

2.1.1. Sulfur-poor Prolamins

The Sulfur-poor prolamins (i.e., ω-gliadins) are characterized by the absence of cysteines and few or no methionines in their primary structure. They have high levels of glutamine and glutamate (40-50 mol%), proline (20-30 mol%) and phenylalanine (7-9 mol%), as reported by Tatham and Shewry (1995). Their

polymorphism has been studied at the protein and DNA levels. In SDS-PAGE electrophoretic studies, each wheat cultivar has been found to have distinct bands, with molecular weights ranging from 44,000 to 78,000. Working at the DNA level, Sabelli and Shewry (1991) used specific probes in combination with aneuploid stocks and estimated the gene copy number between 15 and 18 copies (cv. Chinese Spring).

The S-poor prolamins are coded by genes on the short arm of homoeologous chromosomes 1A, 1B and 1D (Payne, 1987). The loci are designated *Gli-1A, Gli-1B* and *Gli-1D*. These loci also code for the γ-gliadins and are tightly linked to *Glu-3A, Glu-3B* and *Glu-3D*, respectively (Singh and Sheperd, 1988).

2.1.2. Sulfur-rich Prolamins

The S-rich prolamins can be divided into three groups: α-type gliadins (includes α- and β-gliadins), γ-type gliadins and low M_r glutenin (or aggregated-type or LMW). These proteins have 2-3 mol% of cysteine plus methionine (Shewry *et al*, 1986). For the monomeric gliadins, these authors suggested that most of the cysteine residues are involved in **intramolecular** disulphide bonds (reduction causes conformational changes in the molecules, influencing their mobility in the electrophoresis). There are also **intermolecular** disulphide bonds

for the LMW glutenins, which convert these polypeptides into polymeric proteins. This gives rise to large functional differences between the α-/ γ-type gliadins and the LMW glutenins.

Primary structure of gliadin is organized in several domains: a signal peptide (for subcellular localization), N-terminal sequence, a proline-rich repetitive region without cysteines, and the C-terminal part with cysteines (usually six or eight). The α-type gliadins have two extra polyglutamine domains (Kasarda *et al.*, 1984). Okita *et al.* (1985) compared gliadin cDNA clones and concluded that the gliadin gene family has evolved through tandem duplication/ deletion of DNA segments and point mutations. The point mutations kept the percentage of charged aminoacids low, which is possibly an essential feature for the wheat storage proteins.

Sulfur-rich prolamins have 250 to 300 residues and molecular weights ranging from 31,000 to 44,000, as determined by SDS-PAGE. The α-type gliadins (which include α and β-gliadins) are coded by cluster of genes at *Gli-2A, Gli-2B* and *Gli-2D,* on chromosomes 6AS, 6BS and 6DS, respectively. They differ from the γ-gliadins mainly at the N-terminal sequence (Lew *et al*, 1992).

Even with structural similarities, the homology among different α-type and γ-type gliadins is not high. Rafalski (1986) reported 57% homology between α-gliadin gene pW8233 and the γ-gliadin gene B. One important difference is that

the α-type gliadins have in general six cysteine residues and the γ-type have eight cysteine residues. This even number of cysteines allows for the formation of three or four **intramolecular** disulphide bridges. In instances where a point mutation generated an extra cysteine, the odd number of cysteines permits the formation of an **intermolecular** disulphide bridge, incorporating the polypeptide into the glutenin polymer (Kasarda, 1989). These would act as chain terminators, due to the presence of the extra cysteine residue (Lew *et al.*, 1992).

Müller and Wieser (1995) proposed the disulphide bond structure of the α-type gliadins, based on RP-HPLC separation of gliadins, thermolysin digestion, and RP-HPLC separation of the reduced components. It is noteworthy that the arrangement, which is not random, is possibly an important determinant of the three-dimensional structure and hence associated with function.

Most of the γ-type gliadins are coded at the short arms of group 1 chromosomes and their loci designated *Gli-1A*, *Gli-1B* and *Gli-1D* (these are the same loci that code for the ω-gliadins). In addition, the LMW glutenin loci are tightly linked to them, turning these genomic regions into complex loci (Sabelli and Shewry, 1991). In terms of prolamins diversity, they are the most important genomic regions, coding for a large number of proteins that participate in the formation of the gluten complex. Rafalski (1986) cloned two contiguous 4 and 5 kb wheat sequences, each of which contained a γ-type gliadin gene. This was the first prolamin cluster to be cloned, confirming the tight linkage among

prolamin genes at a *Gli-1* locus. It was also reported that one of the genes was not expressed due to a premature stop codons (i.e., a pseudogene).

Wieser *et al.* (1994) used RP-HPLC to quantify the amounts of α-type gliadin, γ-type gliadin and, ω-gliadin in a diverse group of 16 cultivars. The ω-gliadins ranged from 6.2 to 20%, α-type gliadin from 43.9 to 59.9%, and γ-type gliadin from 30.5 to 45.6%. The relationships between gliadin group proportions and quality parameters (baking volume, SDS-sedimentation, Extensograph resistance and extensibility) were considered poor in terms of predictive utility.

2.1.3. The LMW Glutenin Subunits

The Low Molecular Weight Glutenin Subunits (LMW-GS) are sulfur-rich prolamins and include two groups: the B subunits, with molecular weight between 40,000 and 50,000; and the C subunits, with molecular weights between 30,000 and 40,000. These glutenins are coded by genes at *Glu-3A*, *Glu-3B* and *Glu-3D* loci, which are linked to *Gli-1* and located on the short arm of group 1 chromosomes (Singh and Sheperd, 1988). The LMW-GS are similar to the γ-type gliadins and are considered to be a variant form (Okita *et al.*, 1985). They differ from the gliadins in their polymeric function.

Lew *et al.* (1992) used ion-exchange chromatography to purify glutenin (polymeric protein) and then employed RP-HPLC to fractionate the mixture. After N-terminal sequencing of the resulting fractions, it was possible to identify most of the components of the mixture: HMW-GS, LMW-GS, γ-type gliadins and α-type gliadins. The presence of gliadins in the mixture is explained by the occurrence of a point mutation where a serine residue was mutated to a cysteine residue, resulting in an odd number of cysteines in the molecule. This allows for the incorporation of gliadins into the glutenin polymer. These gliadins probably act as chain terminators and constitute most of the C subunits of LMW glutenins. They are called γ-type glutenin and α-type glutenin (D'Ovidio *et al.*, 1995).

The LMW-GS molecule is very similar to the γ-type gliadins, with the N-terminal half of the polypeptide consisting of repetitive sequences (rich in proline, poor in cysteines) and the C-terminal part made of unique sequences (poor in proline, rich in cysteine). There is also a short unique N-terminal region (12 to 14 residues) that may contain a cysteine residue. These proteins usually have eight cysteine residues. The LMW-GS are expected to form disulphide bridges, three intramolecular and one intermolecular which allows for the complete incorporation into the glutenin polymer. It is predicted that the γ-type gliadins form just four intramolecular disulphide bridges (Kasarda, 1989).

Gupta and Shepherd (1990) described the genetic variation in LMW subunits of glutenin in hexaploid wheat. They reported six alleles at *Glu-3A*, nine

alleles at *Glu-3B*, and five alleles at *Glu-3D* using a two-step, one-dimensional SDS-PAGE procedure. Each allele consists of several bands of different electrophoretic mobility. Singh *et al.*(1991) reported a simplified one-step, one-dimensional procedure for the efficient separation of LMW glutenins, without the problem of comigration of gliadins, as these have similar electrophoretic mobility.

2.1.4. The HMW Subunits of Glutenin

The High Molecular Weight Glutenin Subunits (HMW-GS) or HMW prolamins are the most important storage proteins due to their strong effect on breadmaking quality parameters. These are proteins with molecular weights ranging from 67,000 to 88,000. The number of residues varies from 627 up to 809 (Shewry *et al.*, 1992).

The HMW-GS are coded by genes at *Glu-1A*, *Glu-1B* and *Glu-1D*, on the long arm of group 1 chromosomes (Payne *et al.* 1980). Each locus can code for one or two polypeptides: a lower molecular weight x-type and a higher molecular weight y-type. Most of the loci code for a pair of subunits (x- and y-type), but there are few alleles in which the y-type is absent.

Polymorphism is highest at *Glu-1B*, with five common alleles and at least six rare alleles. At *Glu-1D* there are two common alleles and four rare alleles.

The *Glu-1A* locus is the least polymorphic, with just three alleles, where one of them is null and the other two do not present a functional y-type gene (Shewry *et al.*, 1989).

The typical structure of the HMW-GS is a large central repetitive domain flanked by unique N-terminus (81 to 104 residues) and C-terminus (42 residues), with three or five cysteines located in the N-terminal region and one cysteine in the C-terminal region. Some subunits may have an additional cysteine residue in the repetitive region, either close to the N- or C-terminal regions (Shewry *et al.*, 1986).

The repetitive domain is based on three different motifs: the hexapeptide Pro-Gly-Gln-Gly-Gln-Gln (consensus), the nonapeptide Gly-Tyr-Tyr-Pro-Thr-Ser-Pro/Leu-Gln-Gln (consensus), which are present in both types of subunits, and the tripeptide Gly-Gln-Gln, only present in x-type subunits.

The predicted secondary structure for the N- and C-terminal regions are α-helical, while the large central repetitive domains are predicted to form regularly repeated β-reverse turns, that may form a spiral supersecondary structure. It is unclear how important this structure is for the functional properties of these proteins (Miles *et al.*, 1991).

2.2. The Relationship between Prolamins and Quality

2.2.1. The HMW Glutenins and Breadmaking Quality

The determination of the subunit allelic composition of a wheat line can be used to predict breadmaking performance. Payne *et al.*(1979) were the first to study the effects of subunit allelic composition of the HMW glutenins on quality. Their original method involved SDS-PAGE electrophoresis of reduced. They were able to develop the well-known HMW scoring system (Payne *et al.*, 1987), where each subunit pair has a score from one to four, and each line could have an overall score from three to ten, summing across loci. This approach explained 47-60% of the variation in quality among 84 British cultivars. In addition, they were able to improve the scoring system with an adjustment for the presence of a 1BL.1RS translocation. This translocation is associated with poor quality in some genetic backgrounds and reduces the overall score.

Several authors subsequentely characterized the HMW glutenin subunit allelic composition of several cultivars. Countries with a tradition of exporting wheat of good breadmaking quality (e.g., Argentina) reported average scores as high as 9.5, among 23 cultivars. In Great Britain, where quality has not been an important breeding objective for many years, the average score was 5.8 (Payne *et al.*, 1987).

Carrilo *et al.*(1990) developed a set of 48 recombinant inbred lines from a cross between two cultivars with contrasting HMW glutenin subunit alleles. The variation among HMW alleles explained 25% of the variation in SDS-sedimentation volume. A set of 135 random wheat lines was developed from a randomly mated population with the purpose of studying the effects of the HMW glutenins on quality without the confounding effect of linkage disequilibrium (Dong *et al.*,1991). They found significant genetic effects for mixing time at the *Glu-1B* and *Glu-1D* loci and a significant intergenomic interaction between *Glu-1A* and *Glu-1B*, confirming what other research groups had previously found. The subunit pair 6+8 (*Glu-1B*) had a large negative effect on the mixing properties, while the subunits 7+8, 17+18 (*Glu-1B*) and 5+10 (*Glu-1D*) had positive effects on these properties.

The contribution of the HMW glutenin subunits was demonstrated with a group of 70 wheat cultivars (one year's data), using the Alveograph parameters dough strength (W), tenacity (P) and swelling (G). It was shown that subunits 2*, 7+9, and 5+10 have a positive correlation with dough strength and tenacity, while subunits 1, 13+16 and 17+18 have a positive correlation with extensibility (Branlard and Dardevet, 1985).

Brunori *et al.* (1989) studied the progeny of two crosses to demonstrate transgressive segregation for quality due to the recombination of the most favorable HMW alleles. The Alveograph was used to measure quality in this

study. Correlations between dough strength (W) and protein content were positive, in all three years of the experiment.

To correlate several quality parameters (Extensograph resistance and extensibility, loaf volume, SDS-sedimentation value, etc.) to HMW glutenin subunit composition and gliadin allelic composition in Australia, Campbell *et al.*(1987) used a group of 71 cultivars of different origin and found a strong positive effect for the subunit pair 5+10, compared to the pair 2+12. They also found significant differences between the gliadin alleles Gli 58 and Gli 59, demonstrating that there are more factors other than the HMW-GS controlling quality. Their results were confirmed in a companion paper (Cressey *et al.*,1987) with a set of 60 advanced lines from New Zealand.

2.2.2. The LMW Glutenins and Breadmaking Quality

Gupta *et al.*(1989) investigated the effect of allelic segregation in the progeny of the cross Kite / MKR(111/8), using 56 F_2-derived F_6 lines. They studied the *Glu-1A* locus, with alleles *b* and *c* (null), and, for the first time, the *Glu-A3* locus, with allele *m* and a null allele, using a two-step, one-dimensional SDS-PAGE procedure. This procedure allowed for the separation of the LMW-GS from the comigrating gliadins. The Extensograph data revealed large negative effects on the dough properties for Maximum Resistance (R_{max}) and

Extensibility (*Ext.*), due to the presence of the null alleles at both *Glu-1A* and *Glu-3A*. In addition, the effects were additive, which is important in selecting for quality in a breeding program.

A collection of 222 cultivars from 32 countries was studied (Gupta and Sheperd, 1990) using the two-step, one-D SDS-PAGE procedure. It was possible to characterize the existing allelic variation at the LMW-GS loci (*Glu-3A, Glu-3B* and *Glu-3D*). These authors suggested the current LMW-GS nomenclature which include six alleles at *Glu-3A* (*a, b, c, d, e* and *f*), nine alleles at *Glu-3B* (*a* to *i*) and five alleles at *Glu-3D* (*a* to *e*). They also recommended a set of standards for the identification of the LMW-GS alleles.

Two sets of wheat cultivars ("World" and "Australian"), with 48 and 53 genotypes respectively, were employed in order to investigate the relationships between the glutenin allelic compositions and the physical dough properties of the cultivars (Gupta *et al.*, 1991). Extensograph data for the Australian set was obtained from the crop quality surveys for each cultivar. The correlations between predicted and observed Extensograph resistance (R_{max}) values were significant for both HMW and LMW glutenins. The predictive superiority of the LMW-GS in the Australian set was possibly due to the high frequency of the most favorable HMW alleles in that collection. As a result, most of the existing quality variation was explained by the LMW-GS allelic composition. Predictive models were developed with HMW, LMW or both. The best model always

included both HMW and LMW glutenin composition, for both sets. The HMW glutenin composition generally had a larger contribution to the R_{max} values compared to the LMW-GS loci.

The progeny of the cross Halberd and (W1xMMC)/W1/10 was used to study the effect of HMW and LMW-GS allelic variation on quality parameters, using the Extensograph (Gupta *et al.*, 1994). The two lines have distinct alleles at the six glutenin loci, providing an excellent genetic material for an in depth analysis of allelic variation and quality. The trials were conducted at two sites, which produced samples with low (8.8%) and medium (12.2%) average protein contents, broadening the scope of the study. There was no significant interaction effect between site and genotypes, which allowed the pooling of data. Large significant differences in R_{max} between the alleles for *Glu-1B*, *Glu-D1*, *Glu-A3* and *Glu-B3* were found, which were not explained by variation in protein content. The effects of four loci appeared to be additive, although there were significant interactions. Approximately 90% of the variation for this trait (R_{max}) was accounted for by the HMW and LMW glutenin subunits, where 60% was explained by the HMW-GS, 20% by the LMW-GS and 10% by interactions. For Extensibility, only 25% of the variation was accounted for by the glutenins, with significant effects for *Glu-D1* and *Glu-B3*. Protein content accounted for 20% of variation on this trait.

Gupta and MacRitchie (1994) used this same group of lines to investigate the biochemical basis for the allelic differences in dough strength. It was shown that these differences are due to both quantity and quality (polymerizing response) of polypeptides produced by each allele. It was also shown that the effects on breadmaking quality at the short arms of group 1 chromosomes in genomes A, B and D are due to the LMW glutenins, instead of the linked gliadins.

2.2.3. The Effect of the Wheat-Rye Translocations on Quality

The 1BL.1RS and 1AL.1RS wheat-rye chromosomal translocations have been used for many years to increase yield potential, to broaden adaptation and to improve yield stability (Rajaram *et al.*, 1983). Recently a 9% yield advantage for the 1BL.1RS lines was reported (Moreno-Sevilla *et al.*, 1995), using randomly selected F_3-derived lines over seven environments. In contrast to these advantages, wheat-rye translocations are considered to confer inferior breadmaking quality to cultivars (Dhaliwal *et al.*, 1990).

The Australian cultivars Cook, Oxley and Egret with their 1BL.1RS backcross derivatives were used to study the effect of the wheat-rye translocations in several milling and quality parameters (Dhaliwal *et al.*, 1987). There were no significant effects for 1000-grain weight, test weight, grain

protein, flour yield, and farinograph water absorption. However, there were significant negative effects (on hard wheats) for SDS-sedimentation volume and dough development time, and a trend for reduced Extensograph R_{max} and Extensibility. For soft wheats, the only significant negative effect was for Extensibility. It was found that the progenies vary with the best progenies being similar in quality attributes compared to their recurrent parent.

Pena *et al.*(1990) analyzed the 22nd CIMMYT International Bread Wheat Screening Nursery (IBWSN), composed of 295 lines for the presence of 1BL.1RS translocations and their breadmaking quality characteristics. It was not possible to associate inferior quality with the 1BL.1RS translocations. The authors suggested that it is possible to select good breadmaking quality lines carrying a 1BL.1RS translocation.

A series of lines derived from the cross between TX81V6610 (a TAM200 sister line with 1AL.1RS) and Siouxland (1BL.1RS) were developed and characterized for the four possible types of progeny: no translocation (normal), single 1BL.1RS, single 1AL.1RS and double translocation (Graybosch *et al.*, 1993). Significant differences among the classes for most quality parameters were observed, but not for protein content. Means comparisons indicate a ranking in SDS-sedimentation volume: the *normal* lines with the highest volumes and in decreasing order 1AL.1RS, 1BL.1RS, and double translocation. However, the presence of lines which performed similar to the *normal* lines was observed

within the single translocation groups, indicating the possibility of combining the favorable agronomic characteristics of wheat-rye translocation lines with good breadmaking quality.

Recently, a set of 373 advanced bread wheat lines derived from seven crosses between lines with and without the 1BL.1RS translocation was developed by Lee *et al.* (1995). The lines were evaluated for Mixograph parameters and for SDS-sedimentation volume. The deleterious effects of the 1BL.1RS translocation on the quality parameters were confirmed for the populations, although 5% of the 1BL.1RS lines were considered of acceptable quality. For the non-1BL.1RS group, 50% of the lines were considered acceptable. Using SE-HPLC, it was possible to demonstrate that these negative effects on quality are due to a decreased glutenin content and increased levels of salt-water soluble proteins, which are in accordance with the addition of the rye secalin genes and the consequent loss of the LMW glutenin genes.

2.3. Hardness

Kernel hardness can be defined as resistance of the kernel to fracture (Anjum and Walker, 1991). Hard grain wheats are characterized by a continuous protein matrix in which the large and small starch granules are immersed. In soft

wheats there is no continuous protein matrix and the adhesion between protein and starch granules is weak.

Hardness is a very important kernel characteristic as it has an effect on milling and baking properties of wheat and, as a consequence, on marketing value. In terms of milling properties, hardness affects several features including: the energy necessary to fracture the grain; cleanness of separation of the kernel constituents (endosperm and bran), fragment size and its distribution, and the sifting performance of the resulting flour. As a result of these factors, hard wheat flours flow through the mill much better than soft wheats, increasing the overall efficiency of the milling process.

In terms of dough strength, there is a general perception that hard wheats produce stronger doughs than soft wheats. The biochemical basis for this is unclear, but an interesting point is the linkage between the most important gene for hardness (*Ha*) and one of the two genes that affects the levels of free lipids (Morrison, 1989).

Kernel hardness also affects starch damage and availability of fermentable sugars (Pomeranz, 1984), where hard wheat flours have higher starch damage than soft wheat flours as a consequence of the coherent nature of the endosperm (MacRitchie, 1983). Starch damage is important in terms of

water absorption (higher starch damage, higher absorption) and mixing requirements.

Although the environment influences hardness, the kernel texture is genetically controlled. The major *Ha* gene (Hardness) is located in the short arm of chromosome 5D and possibly there are minor genes at 5A and 5B that also affect grain hardness (Law *et al.*, 1978).

Greenwell and Schofield (1986) using SDS-PAGE of water washed starch granules demonstrated that wheat presents a 15 kDa polypeptide band, which is very strong in soft wheats and faint in hard wheats (even absent in durum wheats). This polypeptide was called "grain softness protein", and it is coded in the short arm of chromosome 5D. It may be the product of the *Ha* gene, but that has not been confirmed. This protein probably acts as a non-sticking agent, decreasing the adhesion between the starch granules and the protein matrix, promoting "softness" (Anjum and Walker, 1991).

3- Materials and Methods

3.1- Study "A"

3.1.1. Genotypes and Field Trials

Two spring wheat germplasm pools were studied, representing advanced lines adapted to different ecological regions in Southern Brazil. Identified as Populations 1 and 2, they composed 36 and 40 genotypes, respectively. Both populations included four check cultivars (BR-23, BR-35, BR-18 and Klasic).

The trials were planted in the spring of 1995 and 1996 at the Oregon State University field laboratory at East Farm, near Corvallis, OR. The soil type at the site is a Chehalis silt loam. A randomized complete block design (RCBD) with two replications was employed (Population 1/95 trial had three replications). Each plot consisted of six 2.5-m rows with 0.25 m between rows, seeded at 130 kg ha^{-1}(for 1995, the Population 1 trial was planted in six 5-m rows with 0.25 m between rows). In 1995, the trials were planted on April 25th and prior to planting 56 kg of N ha^{-1} and 40 kg of P_2O_5 ha^{-1} were incorporated into the seed bed. The trials were top-dressed at heading stage (Feekes stage 10.1) with 80 kg of N ha^{-1}. In 1996, the trials were planted on April 7th, fertilized with 150 kg of P_2O_5 ha^{-1} and top-dressed with 100 kg of N ha^{-1}, in two applications: the first at Feekes

stage 3 and the second at Feekes stage 6. The trials were irrigated to avoid water stress. Climatological records for these trials are presented in Appendix Tables 14 and 15. Weeds were controlled using Thifensulfuron (0.027L A.i. ha^{-1}) and Diclofop Methyl (1.1L A.i. ha^{-1}) in 1995 and only Thifensulfuron in 1996. Hoeing was also employed in both years. The use of fungicides was not necessary in either year.

3.1.2- Breadmaking Quality Data

Five-hundred gram samples from each plot were used for quality evaluation. Protein percentage and hardness were determined on wholemeal flour by near-infrared reflectance spectroscopy using a Technicon Infralyzer 400. SDS-sedimentation volume was determined using the procedure proposed by Axford *et al.* (1979) with slight modifications: a 3.2 gram wholemeal flour sample was used and the tests were run at room temperature. The samples for the Alveograph tests were milled in a Brabender Quadrumat Senior, after tempering overnight. Dough properties were evaluated using the Chopin Alveograph apparatus (AACC Method 54-30A). The variables associated with dough strength, tenacity and extensibility were determined. Dough strength, or Deformation Energy, is measured by the value W and represents the energy used to inflate a bubble until its rupture, in 10^{-4} x J. It is calculated from the area under the curve (S, cm^2), using the formula $W = 6.54 \times S$. Tenacity is measured

by the value P and represents the maximum pressure (in millimeters of water) obtained during the inflation of a dough bubble. Extensibility is measured by the value L and represents the length (in millimeters) of the curve, from the point where the inflation of the bubble starts until the point where it bursts (Faridi and Rasper, 1984)

3.1.3- Electrophoretic Analysis

The High Molecular Weight (HMW) and Low Molecular Weight (LMW) glutenin subunit allelic composition of the lines were determined using the Singh procedure (Singh *et al.*, 1991). The nomenclature of Payne and Lawrence (1983) was used for HMW-GS and the nomenclature described by Gupta and Sheperd (1990) was used for the LMW glutenin subunits (LMW-GS).

Electrophoresis was carried out using Bio-Rad vertical dual slab gels (160x140x1.5mm) with 20 wells. For the separating gels a 12.5% (w/v) acrylamide concentration with 1.5% crosslinker concentration (bis-acrylamide / acrylamide), with 0.1% (w/v) SDS and 0.375M Tris-HCl, pH 8.8, was used. The stacking gels contained 3% (w/v) acrylamide, 2.7% crosslinker, 0.1% (w/v) SDS, 0.125M Tris-HCl (pH 6.8). The gels were polymerized with TEMED and ammonium persulfate. The sample wells were loaded with 15μl of sample (for gliadin gels 20μl). A constant current of 40 mA / gel was applied for 3 to 4 hours,

with cooling by water circulation at 22°C. Gels were stained with Coomasie Blue R-250 for 20 hours and then destained with methanol (25%), according to Neuhoff *et al.*(1980). The extractions were done according to Singh *et al.*(1991), where the gliadins are extracted first, at 50% propanol and the glutenins are extracted later with dithiothreitol (Bio-Rad Laboratories).The glutenins were alkilated with 4-vinylpyridine (Sigma Chemical Co.). Glutenins and gliadins were run in different gels, where glutenin gels were used to classify the lines for their HMW and LMW glutenin subunit allelic composition. The gliadin gels were used to confirm the allelic composition at *Glu-3B* (linked to ω-gliadins) and to verify the presence of secalins (indication of wheat-rye translocation).

3.2- Study "B"

This study was undertaken in order to verify the results of Study "A". Two groups of recombinant inbred lines (RILs) were developed from the crosses BR-35 x ORL-9127 (B1) and ORL-9285 x ORL-92146 (B2). The spring wheat ORL-9127 is a sister line of the cultivar OR-1, with the following allelic composition: *Glu-1A*$^{(a)}$, *Glu-1B*$^{(b)}$, *Glu-1D*$^{(d)}$, *Glu-3A*$^{(d)}$, *Glu-3B*$^{(f)}$ and *Glu-3D*$^{(a)}$.

The crosses were made in Passo Fundo, RS, Brazil, in the spring of 1993. The progeny were planted and sixty F_2 plants were randomly selected from each cross and sent to Oregon in December, 1994. The populations were advanced

using single-seed-descend (SSD) from F_3 to F_5 in greenhouses. One spike per plant per generation was harvested. The seed was subjected to 37°C for seven days before planting to break dormancy. In the spring of 1996, F_6 seed (bulk of spikes from an F_5 plant) were planted in two separate trials on Oregon State University field laboratory at East Farm, near Corvallis, OR. A randomized complete block design with two replications was employed. The plot size was one 0.5-m row, with 0.25 m between rows. The trials were planted on May 5th, 1996 and fertilized with 150 kg of P_2O_5 ha^{-1}. The trials were top-dressed with 100 kg of N ha^{-1} divided in two applications: emergence (Feekes stage 1) and tillering (Feekes stage 4). Weeds were controlled by hoeing.

Each plot was harvested manually and threshed in a plant thresher. Ten gram samples were ground in a Udy Cyclone mill. Protein percentage and hardness were determined by near-infrared reflectance spectroscopy. The breadmaking properties were estimated using SDS-sedimentation test, as described earlier. The electrophoretic banding patterns were determined using the same procedures as in the previous study.

3.3. Study "C"

This study was undertaken to evaluate the impact of a wheat-rye translocation (1AL.1RS or 1BL.1RS) on the dough viscoelastic properties of

wheat. The experimental material employed for this study were the cultivars BH-1146 (Brazil), Jupateco (Mexico) and Hartog (Australia) with their backcross derivatives: BH-1146*6/ Alondra, Jupateco*3/ Amigo and Jupateco*6/ Amigo, Hartog*4 / Skorospelka-35 and Hartog*4 / Amigo. The material was divided into three separate experiments: C1, C2 and C3, respectively, and grown in 1995 and 1996. Each line was compared to its backcross derivatives to evaluate the effect of the translocations on breadmaking quality traits.

For 1995, three trials were hand planted in six 4.0-m row plots, with 0.25 between rows, at a seeding rate of 40 kg ha^{-1}. These trials were managed as previously described. A split-plot design with four replications, with Nitrogen as the whole plot factor and lines as subplots was used for Experiment C1. The two Nitrogen treatments were the control and the addition of 50 kg of N ha^{-1} at heading stage (Feekes stage 10.1). For experiments C2 and C3, a randomized complete block design (RCBD) was employed, with four replications. For 1996, the three trials were planted with a plot drill. Each plot consisted of six 5.0-m rows with 0.25 m between rows, at a seeding rate of 130 kg ha^{-1}.. The trials were conducted as described for Study "A". The breadmaking quality evaluation used is described in section 3.1.2.

3.4- Statistical Analysis

Statistical analysis was conducted using SAS-GLM (SAS Institute Inc., 1995) procedures in order to investigate the relationships between the glutenin subunit allelic composition and SDS-sedimentation, Alveograph dough strength (W), tenacity (P), and extensibility (L). Protein percentage was used as a covariate.

For study "A", the independent variable genotypes (G) refers to the different arrangement of alleles at the six glutenin loci and to classification of kernel hardness, as the lines were classified as having hard of soft grain. The independent variable lines nested in genotypes (L:G) reflects the variation between lines with the same arrangement of glutenin alleles and hardness classification. The model consisted of genotypes, lines nested in genotypes, years (Y), replications nested in years (R:Y), protein percentage and the interactions Y x L:G and Y x G. A second analysis was conducted where instead of using genotypes, it was included the six glutenin loci, hardness and their interactions as independent variables.

A mixed model was used, where genotypes were considered fixed effects and the other five effects (L:G, Y, R:Y, YxL:G and YxG) were considered random effects. The effects of years and replications nested in years are considered random samples from the population of years and replications, as well as the

effect of lines nested in genotypes is a random sample from the population of lines with the same genotype (HMW, LMW and hardness). The tests of hypothesis were based on the Type III Expected Mean Squares. The expected mean square for genotype includes the error variance, GxY variance, L:GxY variance, L:G variance and the fixed effect of genotype. The mean square for genotype was tested with the following denominator: error variance, GxY variance, L:GxY variance and L:G variance, with the appropriate coefficients. The mean square for lines nested in genotype (L:G) was tested with the following denominator: L:GxY variance and error variance, with appropriate coefficients.

The separate analysis for both Populations 1 and 2 allowed for the comparison of the proportion of the variation explained by the different components involving the two germplasm pools. A model with the six glutenin loci, hardness, protein, replications and years was used to estimate the least square (LS) means for the different alleles at each locus, for SDS and dough strength. Least square means were used to account for variation in protein content and the unbalanced genetic composition of the two populations. Accordingly to the number of alleles at the six glutenin loci and hardness classification, there were more than three thousand possible genotypes, for populations with 36 and 40 lines.

For Study "B", a similar model was used, without the effect year, as the experiments were conducted just in 1996. The comparisons between alleles at a locus were accomplished using the estimated least square means. Least square means were used to adjust for protein percentage and to adjust for the unbalanced nature of the factor genotypes (G). These comparisons were used to verify the ranking of the different alleles for SDS-sedimentation obtained from the former experiment.

In Study "C", experiments C2 and C3, the comparisons between lines with and without the translocation were accomplished using a model with the effects lines (L), replications nested in years (R:Y), years (Y), interaction lines x years and the covariate protein percentage, where lines are considered fixed effects and the others are considered random effects. Means were calculated for SDS, W, P, L and protein percentage and compared using the Least Significant Difference Test.

For experiment C1, where a split plot design was used, nitrogen (N) was the whole plot factor and lines (L) was the subplot factor. The effects Lines, Nitrogen and their interaction were treated as fixed effects. Years (Y), replications nested in years (R:Y), the interactions of nitrogen with replications (NxR:Y), Year with Lines (YxL), Year with Nitrogen (YxN) and the three-way interaction Year with Lines with Nitrogen were all considered as random variables. The covariate protein percentage was not included in the model due

to the effect of the main plot factor treatment over protein content. The *F*-tests were accomplished using the "random" statement with the "test " option in the SAS-GLM procedure. Means were calculated for SDS, W, P, L and protein percentage and compared using the Least Significant Difference.

4. Results and Discussion

The major determinants of breadmaking quality in wheat include the amount of protein in the flour, protein quality (i.e., the glutenin allelic composition) and kernel hardness. The amount of protein is determined by agronomic practices, genetics and environment, while hardness is largely an inherited trait. Wheat kernels can be identified into hard and soft types depending on the endosperm characteristics. The glutenin allelic composition explains an important part of the variation in dough quality within specific wheat germplasm. Within glutenins, it is the HMW glutenins contribution that has been studied extensively by research laboratories around the world. Consequently, the existing genetic variation with the HMW glutenins has been also used intensively in order to improve breadmaking quality. However, the contribution of the Low-Molecular-Weight glutenin allelic composition to genetic variation in dough quality is not well established. Their importance has been demonstrated using the Extensograph to measure quality, in Australian germplasm, but the relative ranking of the different alleles at each locus has not been fully investigated (Gupta *et al.*, 1991). Therefore, it is important to determine if a better understanding of the breadmaking quality of wheat can be achieved through the detailed assessment of the contribution of the different alleles at each LMW loci. The effect of the 1B.1R wheat-rye translocation, which involves a *Glu-3* locus, was also investigated as the corresponding LMW glutenins are replaced by secalins.

In order to provide more information as to the contribution of LMW glutenin subunit allelic composition and two wheat-rye translocations to breadmaking quality, three different studies involving different wheat populations were conducted over a three year period.

4.1- Comparisons of Populations (Study "A")

A linear relationship between end product quality value and the measured quality variables is necessary for the elaboration of a strong model that could identify the relative importance of the several factors that affect quality, as a linear model was assumed. The Alveograph parameter W (Dough Strength) and the SDS-Sedimentation Volume have this association (Faridi and Rasper, 1987; Axford et al., 1979). The Alveograph W value is used for grade wheat quality in some countries (e.g., Brazil and Argentina) which determines market value. The SDS-Sedimentation test is used to estimate dough strength in early breeding generations, due to its simple requirements and strong positive correlation with breadmaking quality. Tenacity and extensibility are components of dough strength and are associated with mixing properties, therefore important in the baking industry. The ratio tenacity / extensibility is an important indicator of the equilibrium between these two parameters, where doughs with higher ratios are considered unbalanced and have poor mixing properties.

Both populations had similar means for dough strength, tenacity, extensibility and SDS-sedimentation (Tables 1 and 2). Large differences between years were observed. There was a wide range for all traits, with most observations of dough strength between the values 100 and 400.

The decrease in the mean values for SDS and dough strength from 1995 to 1996 indicates a reduction in breadmaking quality. This was due to the large reduction in protein percentage in 1996 (Table 3). This provided the opportunity to study the allelic variation contribution to quality under a broad range of protein percentage. It is important to note that protein quantity is influenced by environment and agronomic practices which can mask the genetic component.

In Tables 4 and 5, Pearson correlation coefficients between traits reflect the quantitative importance of protein percentage on the quality factors under study. There was a significant positive correlation ($P<0.05$) between W (dough strength) and protein percentage ($r = 0.33$ and $r = 0.34$, for Populations 1 and 2, respectively) when the protein percentage was above 13% (1995). Around 10% protein (1996), the same correlations were $r = 0.52$ and $r = 0.54$. These higher correlation coefficient values indicate that at lower protein levels the expression of dough strength is adversely affected, preventing an acceptable evaluation of the genotypes. It suggests that testing for dough strength should be done at higher protein levels, where the expression of the glutenin genotype is

Table 1- Means, standard deviations, maximum and minimum values for SDS-Sedimentation value and Alveograph Dough Strength (W) for Populations 1 and 2 grown in 1995 and 1996 at the East Farm.

	SDS (ml)				W ($x10^{-4}J$)			
	1995		*1996*		*1995*		*1996*	
	1	2	1	2	1	2	1	2
Mean	44.1	44.1	27.5	29.7	220	228	164	165
St. Deviation	8.5	6.3	6.6	5.3	93	66	50	44
Max.	66	62	41	45	467	424	290	300
Min.	22	33	15	19	62	122	80	100

Table 2- Means, standard deviations, maximum and minimum values for Alveograph Tenacity(P) and Extensibility (L) for Populations 1 and 2 grown in 1995 and 1996 at the East Farm.

	P (mm)				L (mm)			
	1995		*1996*		*1995*		*1996*	
	1	2	1	2	1	2	1	2
Mean	63	63	94	92	134	132	56	52
St. Deviation	21	12	34	17	31	21	22	13
Max.	125	93	154	149	215	171	118	91
Min.	22	42	43	61	69	80	22	22

Table 3- Means, standard deviations, maximum and minimum values for Protein Percentage for Populations 1 and 2 grown in 1995 and 1996 at the East Farm.

	Protein %			
	1995		1996	
	1	2	1	2
Mean	14.0	13.2	10.0	9.4
St. Deviation	1.17	1.15	0.75	0.76
Max.	16.9	16.1	12.1	11.1
Min.	11.0	11.1	8.8	7.8

Table 4. Pearson correlation coefficients (*r*) for Alveograph Dough Strength (W), Tenacity (P), Extensibility (L), Ratio Tenacity / Extensibility (P/L), SDS-Sedimentation Value and Protein Percentage from Population 1 experiments conducted in 1995 and 1996. Values from 1995 are given above the diagonal (upper right) and values from 1996 are given below the diagonal (lower left).

	W	P	L	P/L	SDS	Protein %
W		0.83***	-0.14	0.57***	0.82***	0.33*
P	0.64***		-0.60***	0.92***	0.58***	0.35*
L	-0.03	-0.71***		-0.80***	0.15	-0.13
P/L	0.30	0.91***	-0.81***		0.29	0.31
SDS	0.87***	0.65***	-0.12	0.36*		0.45**
Protein %	0.52***	0.32*	0.13	0.11	0.45**	

*, **, *** Significant at the 0.05, 0.01 and 0.001 probability levels.
(*n*=36)

Table 5. Pearson correlation coefficients (*r*) for Alveograph Dough Strength (W), Tenacity (P), Extensibility (L), Ratio Tenacity / Extensibility (P/L), SDS-Sedimentation Value and Protein Percentage from Population 2 experiments conducted in 1995 and 1996. Values from 1995 are given above the diagonal (upper right) and values from 1996 are given below the diagonal (lower left).

	W	P	L	P/L	SDS	Protein %
W		0.80***	0.14	0.41**	0.78***	0.34*
P	0.54***		-0.42**	0.85***	0.42**	0.16
L	0.56***	-0.37*		-0.80***	0.46**	0.26
P/L	-0.11	0.74***	-0.83***		-0.01	-0.05
SDS	0.89***	0.40**	0.55***	-0.21		0.45**
Protein %	0.54***	0.23	0.38*	-0.11	0.41**	

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.
(*n*=40)

enhanced. The effect of the protein percentage was accounted for with the estimation of least square means for the comparisons between alleles.

The SDS-sedimentation correlated significantly with Alveograph dough strength (r = 0.77 to r = 0.88) indicating that it can be employed to measure dough strength and used in early generation testing in a breeding program (Axford *et al.*, 1979; Lorenzo and Kronstad, 1987, O'Brien and Ronalds, 1987). A positive correlation between SDS and protein percentage was also detected.

Frequency distributions for dough strength for lines from Populations 1 and 2 are presented on Figures 1 and 2. The large decrease in dough strength associated with the decrease in protein percentage from 1995 to 1996 is evident. The existing range in quality is also evident, with few lines in the extremes and most the lines concentrated towards the mean values for each year.

Combined analysis of variance for Populations 1 and 2 are presented in Tables 6 and 7, respectively. The term genotype in the tables refers to different arrangements of alleles at the six glutenin loci. As an additional factor kernel hardness was included with lines noted as hard or soft. Lines with the same combination of alleles within a hardness class are expected to perform alike, if these seven factors control most of the variation in breadmaking quality.

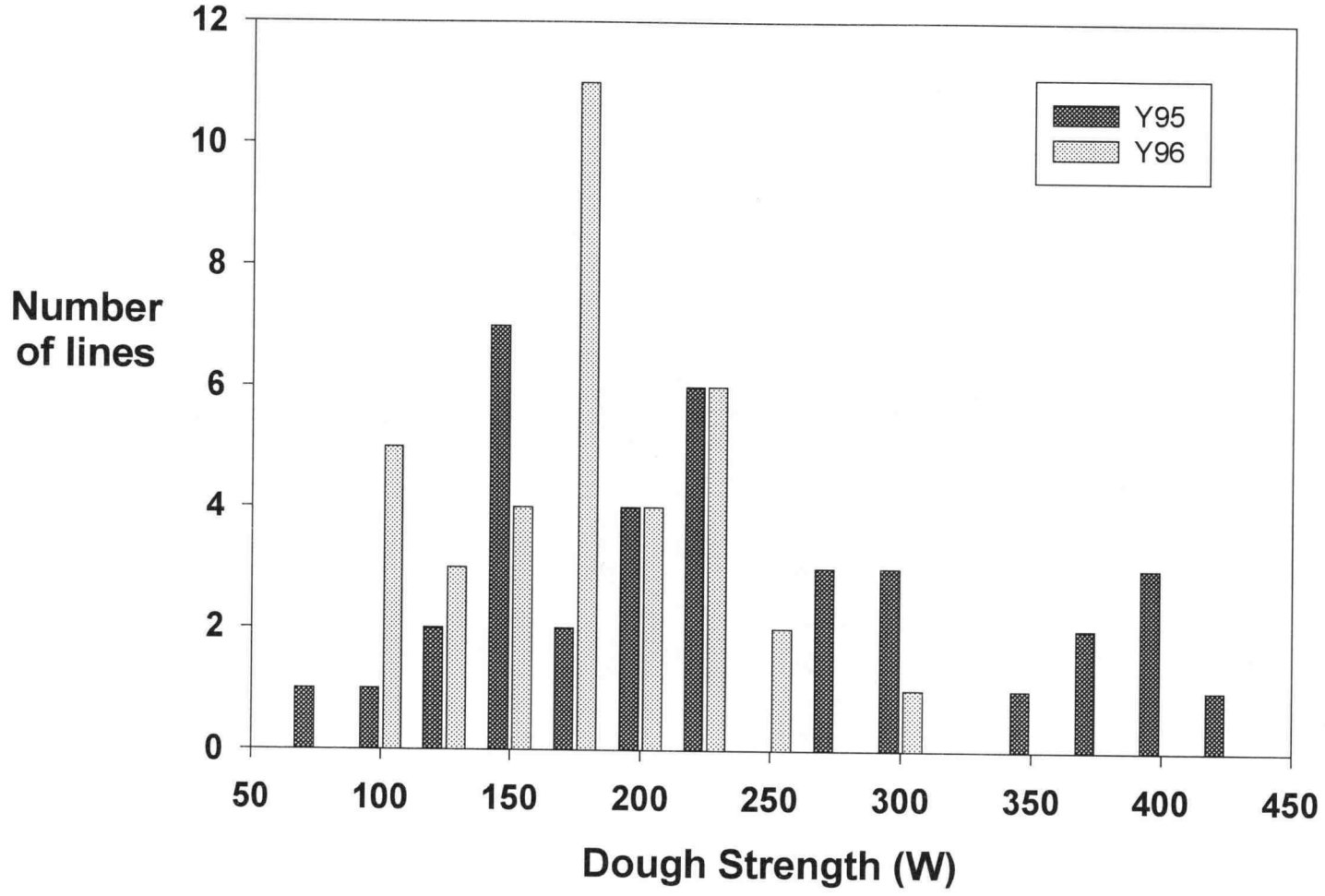

Figure 1- Frequency distribution for average dough strength of lines from Population 1 on 1995 and 1996.

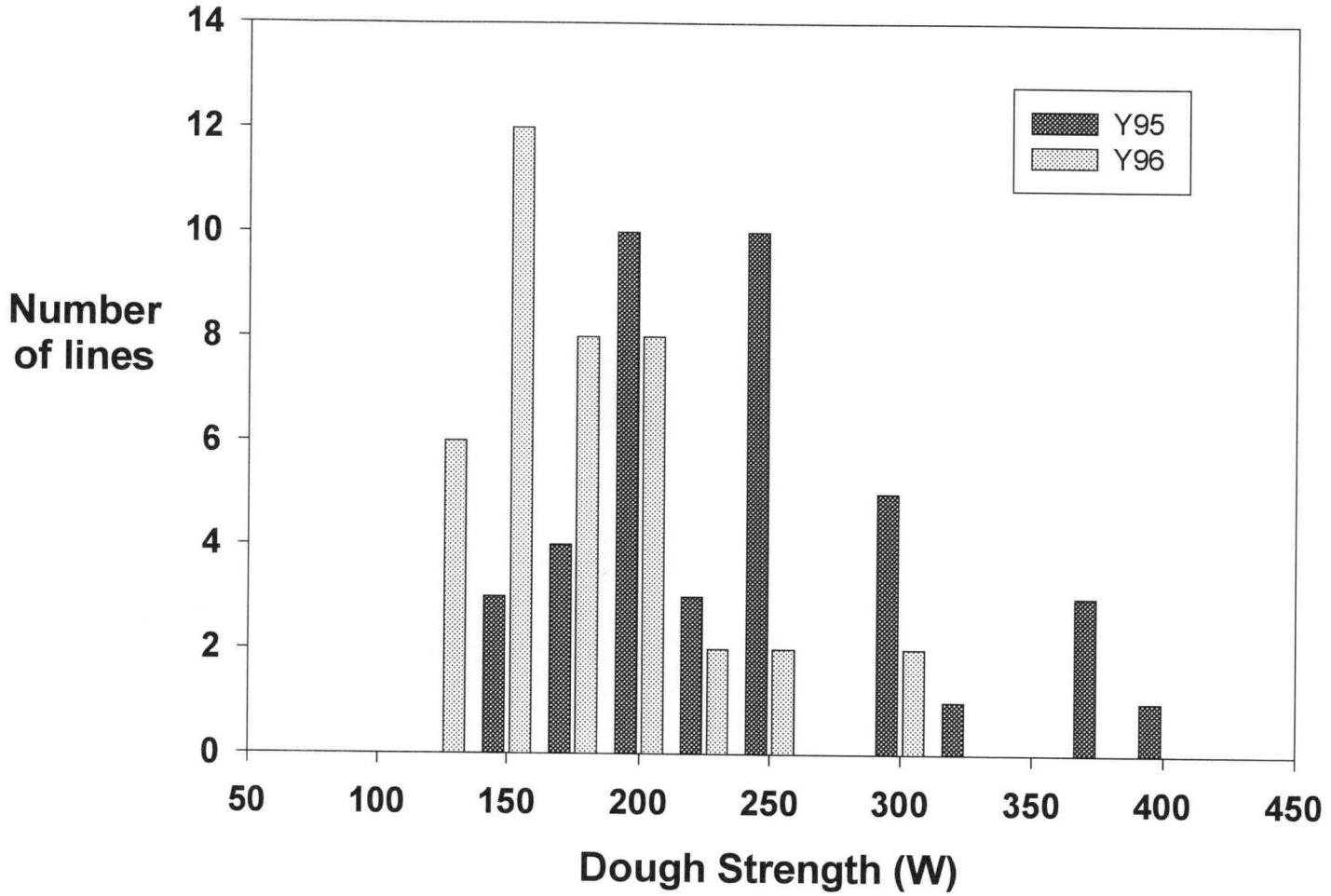

Figure 2- Frequency distribution for average dough strength of lines from Population 2 on 1995 and 1996.

Table 6- Mean Squares for SDS-Sedimentation, Dough Strength (W), Tenacity (P) and Extensibility (L) for Population 1 in 1995 and 1996:

Source:	df	SDS	W	P	L
Genotypes	29	230.4***	22188**	3524*	3236
Glu-1A†	2	19.9	13825	4389	2014
Glu-1B	3	43.9	28422*	10172**	7001
Glu-1D	1	14.0	33664*	3485	859
Glu-3A	3	187.2**	13795	5254*	2706
Glu-3B	5	161.9**	12848	25	807
Glu-3D	2	70.8	19597	214	718
Hardness	1	148.1*	901	1231	377
Glu-3A x *Glu-3B*	2	11.7	15017	4775*	3747
Glu-1D x *Glu-3B*	2	112.5	24075*	4381	2035
Glu-1D x Hardness	1	66.9	21120	6953*	3594
Glu-3B x Hardness	1	123.6	19459	5073	2861
Glu-3A x Hardness	2	36.5	4360	314	527
Glu-1A x *Glu-3A*	1	39.8	1742	2224	1302
Glu-3D x Hardness	1	10.4	11226	1807	286
Protein	1	307.2***	4155***	31	1876***
Replications (Year)	3	12.0**	231	18	224*
Year	1	28.6**	151	1345**	3788***
Lines (Genotypes)	6	16.7	2356	826**	1628
Year x Lines(Genotypes)	6	5.1	879**	72***	481***
Year x Genotypes	29	22.3*	5062*	528**	358
Error	104	2.42	275	10.6	76.6
CV%		4.2	8.4	4.3	8.5

†- *Glu-1A*, *Glu-1B* and *Glu-1D* are HMW loci, *Glu-3A*, *Glu-3B* and *Glu-3D* are LMW loci.
*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.

Table 7- Mean Squares for SDS-Sedimentation, Dough Strength (W), Tenacity (P) and Extensibility (L) for Population 2 in 1995 and 1996:

Source:	df	SDS	W	P	L
Genotypes	26	136.5***	11251**	836*	845*
Glu-1A†	2	45.4	3259	864	881*
Glu-1B	2	9.6	2813	976	180
Glu-1D	1	25.2	277	1	70
Glu-3A	3	231.4***	14244**	442	238
Glu-3B	5	241.1***	19959***	398	1839***
Hardness	1	34.7	190	1	47
Glu-3A x *Glu-3B*	2	2.0	209	624	831*
Glu-1B x *Glu-3B*	4	19.0	2734	1006*	676*
Glu-1D x *Glu-3A*	1	255.4**	20415**	659	126
Glu-1A x *Glu-3A*	2	6.3	1925	279	49
Protein	1	191.3***	8309***	76	602
Replications (Year)	2	33.1***	317	13	133
Year	1	16.6	34	1816***	5169***
Lines (Genotypes)	13	14.2**	1499	254*	151*
Year x Lines(Genotypes)	13	4.2	1277***	83***	52
Year x Genotypes	26	7.2	1845	180	320***
Error	77	2.9	285	24.8	167.2
CV%		4.6	8.6	6.4	14.1

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.
†- *Glu-1A*, *Glu-1B* and *Glu-1D* are HMW loci, *Glu-3A* and *Glu-3B* are LMW loci.

Mean squares for genotypes were significant for both SDS-sedimentation and dough strength, in both populations. This indicated the presence of differences in dough quality among the different combinations of alleles.

The term lines nested in genotypes (L:G) reflected the variation between lines with the same arrangement of glutenin alleles and hardness. For the trait dough strength, this component was found to be non-significant for both populations, indicating that lines with the same arrangement of alleles (HMW and LMW) and hardness responded alike. This suggested that most of the factors that control dough strength in these populations were identified. In contrast, the contribution of L:G to tenacity was found to be significant for both populations, indicating that there are factors other than those measured controlling this property.

The effect of protein percentage was evident for SDS and dough strength, in both populations, demonstrating the important contribution of protein content to breadmaking quality. It is important to consider that the glutenin genotypes did not influence the variation in protein percentage, an assumption for the use of analysis of covariance. For Population 1, the contribution of protein content to variation in extensibility was found to be significant as well.

The large difference in protein percentage between the two years could have enhanced the effect of years and its interactions to the variation in quality.

The variation due to years was significant for tenacity and extensibility in both populations. This suggests that protein percentage is not the only source of variation for these traits between years within genotypes. In contrast, a significant effect of years for dough strength was not found. The component year x genotypes was significant for SDS and dough strength only for Population 1 ($P < 0.05$). This suggests that some genotypes (six glutenin alleles and hardness) may respond differently to the year to year variation in protein percentage, whereas the response in dough strength with variation in protein percentage is uniform for Population 2 genotypes. These can be due to the presence of genotypes that are non-responsive to increases in protein content. Years by lines nested in genotype interactions were found to be significant for dough strength and tenacity in both groups. This indicates that there are significant differences in the quality performance of lines with the same arrangement of alleles from one year to the other, even accounting for the effect of protein content.

Coefficients of variation were low for most of the variables. It is noteworthy that the lowest coefficients were for SDS-sedimentation for both populations, possibly to the simple and direct nature of the test.

4.1.1. The HMW Glutenins Contribution

A contribution by locus *Glu-1A* to the variation in dough quality was not observed except for extensibility in Population 2 (Table 7). This would be expected due to the high frequency of alleles *Glu-1A*$^{(a)}$ or *Glu-1A*$^{(b)}$ in both populations, which are considered to have similar contributions to the overall quality. Alleles *Glu-1A*$^{(a)}$ and *Glu-1A*$^{(b)}$ had a similar effect and allele *Glu-1A*$^{(c)}$ was inferior to both in this study (Tables 8 and 9).

Alleles *Glu-1B*$^{(b)}$, *Glu-1B*$^{(c)}$ and *Glu-1B*$^{(i)}$ were detected at *Glu-1B* locus in Populations 1 and 2. In addition, allele *Glu-1B*$^{(f)}$ was found among some lines in Population 1. There is evidence for a significant contribution of *Glu-1B* locus to the variation in dough strength, tenacity and extensibility for Population 1 (Table 6). The LS means ranking for this population indicated that alleles *Glu-1B*$^{(b)}$ and *Glu-1B*$^{(c)}$ are superior to allele *Glu-1B*$^{(i)}$ in terms of dough strength (Table 8). No significant contribution of this locus on any quality trait was noted on Population 2. The LS means for SDS-sedimentation reflected a similar contribution of the three alleles (Table 9). Payne *et al.* (1984) considered these alleles advantageous to breadmaking quality, in relation to other *Glu-1B* alleles (e.g., *Glu-1B*$^{(a)}$, *Glu-1B*$^{(d)}$ and *Glu-1B*$^{(e)}$).

Table 8- Least Square Means for SDS-Sedimentation Value and Alveograph W (Dough Strength) for the different alleles at the High-Molecular-Weight Glutenin loci for Population 1.

Glu-1A			*Glu-1B*			*Glu-1D*		
Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)
a	$36.0^{a\dagger}$	189^{c}	b	33.0^{f}	193^{g}	a	32.9^{k}	136^{m}
b	35.0^{a}	182^{c}	c	35.5^{e}	202^{g}	d	35.8^{j}	212^{l}
c	32.0^{b}	152^{d}	f	37.8^{e}	172^{gh}			
			i	31.0^{f}	130^{h}			

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 9- Least Square Means for SDS-Sedimentation Value and Alveograph W (Dough Strength) for the different alleles at the High-Molecular-Weight Glutenin loci for Population 2.

Glu-1A			*Glu-1B*			*Glu-1D*		
Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)
a	$36.0^{a\dagger}$	211^{c}	b	36.5^{e}	219^{f}	a	34.7^{h}	182^{i}
b	36.2^{a}	200^{c}	c	34.2^{e}	165^{g}	d	35.4^{h}	199^{i}
c	32.9^{b}	161^{d}	i	34.5^{e}	188^{f}			

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

Glu-1D is considered the most important locus in influencing breadmaking quality (Dong *et al.*, 1991, Lookhart *et al.*, 1993, Gupta *et al.*, 1994). There was evidence for a significant contribution in dough strength for Population 1 (Table 6) and a large difference in LS means for dough strength between alleles was detected for this group (Table 8). A difference was not observed in SDS-sedimentation, even though allele *Glu-1D*$^{(d)}$ was superior to *Glu-1D*$^{(a)}$ (35.8>32.9, $P = 0.0019$). For Population 2, the *Glu-1D* locus contribution was non-significant for all traits and no differences in LS means were detected (Tables 7 and 9). Conflicting results are found in the literature, where several groups demonstrated the superiority of *Glu-1D*$^{(d)}$ allele (Payne *et al.*, 1984, Branlard and Dardevet, 1985, Pogna *et al.*, 1989) while another group (Gupta *et al.*, 1991) found these alleles to be similar in their contribution in some genetic backgrounds.

The results obtained in this study for the three HMW glutenin loci are in accordance with what is reported in the literature, attesting that the methodology used was correct. This fact was important when the contribution of the LMW glutenin loci was studied, considering the lack of information about these three multiallelic loci.

4.1.2. The LMW Glutenins Contribution

Alleles *Glu-3A*$^{(b)}$, *Glu-3A*$^{(c)}$, *Glu-3A*$^{(d)}$ and *Glu-3A*$^{(e)}$ were observed in different lines at the LMW *Glu-3A* locus in both Populations 1 and 2. There is evidence for a significant contribution of this locus to differences in SDS, for both populations (Tables 6 and 7). A significant effect for tenacity was also detected for Population 1 (Table 6), while a significant effect for dough strength was found for Population 2 (Table 7). The Least square mean comparisons were consistent across groups for SDS and dough strength (Tables 10 and 11), where the relative ranks among alleles were similar for both populations. This indicated that allele *Glu-3A*$^{(d)}$ was superior for SDS and W in relation to the other alleles, with allele *Glu-3A*$^{(e)}$ being the most inferior. Allele *Glu-3A*$^{(e)}$ was expected to be inferior to others as it is a null allele (a null allele does not contribute with any polypeptide to the glutenin polymer).

The *Glu-3B* locus was the most polymorphic, with five possible glutenin alleles (*Glu-3B*$^{(b)}$, *Glu-3B*$^{(d)}$, *Glu-3B*$^{(f)}$, *Glu-3B*$^{(g)}$, and *Glu-3B*$^{(h)}$) and a wheat-rye translocation (1BL.1RS) that includes this locus. The effect of the translocation on the flour properties was substantial and will be discussed in a later section. Differences due to the *Glu-3B* allelic composition were found for SDS for Population 1 (Table 6). In Population 2, differences for SDS, dough strength and

Table 10- Least Square Means for SDS-Sedimentation Value (ml) and Alveograph W (Dough Strength) for the different alleles at the Low-Molecular-Weight Glutenin loci for Population 1.

Glu-3A			***Glu-3B***			***Glu-3D***		
Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)
b	$29.4^{c\dagger}$	135^{f}	*b*	41.4^{g}	265^{m}	*a*	36.8^{p}	203^{r}
c	36.1^{b}	183^{e}	*d*	34.0^{hi}	210^{mn}	*b*	29.5^{q}	104^{s}
d	41.4^{a}	238^{d}	*f*	35.5^{h}	166^{n}	*c*	36.6^{p}	216^{r}
e	30.5^{c}	141^{f}	*g*	24.8^{k}	38^{o}			
			h	38.6^{gh}	216^{m}			
			1B.1R	31.7^{i}	149^{n}			

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 11- Least Square Means for SDS-Sedimentation Value and Alveograph W (Dough Strength) for the different alleles at the Low-Molecular-Weight Glutenin loci for Population 2.

Glu-3A			***Glu-3B***		
Allele	SDS (ml)	W ($x10^{-4}J$)	Allele	SDS (ml)	W ($x10^{-4}J$)
b	$30.6^{c\dagger}$	189^{de}	*b*	42^{f}	259^{j}
c	35.1^{b}	175^{e}	*d*	40.5^{fg}	224^{k}
d	41.6^{a}	229^{d}	*f*	35.5^{h}	186^{k}
e	33.0^{c}	170^{e}	*g*	25.5^{i}	124^{l}
			h	37.7^{g}	189^{k}
			1B.1R	29.0^{i}	162^{l}

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

extensibility were detected (Table 7). The effect of this locus was significant across years and germplasm groups.

Allele *Glu-3B*$^{(b)}$ was superior in both populations, followed by *Glu-3B*$^{(d)}$, *Glu-3B*$^{(f)}$ and *Glu-3B*$^{(h)}$ (Tables 10 and 11). The superiority of allele *Glu-3B*$^{(b)}$ was also demonstrated by Gupta *et al.* (1991 and 1994) using an Extensograph. The latter three alleles were superior to the 1BL.1RS translocation (Tables 12 and 13). This is likely due to the fact that the translocations involve a large segment of the short arm of the 1B chromosome, where the *Glu-3B* and *Gli-1B* loci are located. The replacement of glutenins and gliadins by the rye secalins is the possible cause for the differences in the dough quality, as the these secalins apparently do not polymerize and consequently do not contribute to the gluten polymer (Shewry and Tatham, 1990).

Few polymorphisms were found at the *Glu-3D* locus (*Glu-3D*$^{(a)}$, *Glu-3D*$^{(b)}$ and *Glu-3D*$^{(c)}$), with allele *Glu-3D*$^{(a)}$ being present in most of the lines in Population 1 and monomorphic in Population 2. This suggests that there is a large potential for improvement if alleles other than a are introgressed into these germplasm groups. Gupta and Sheperd (1990) reported the existence of five alleles at this locus. In order to illustrate this possibility, the allelic composition of cultivars of excellent breadmaking quality can be examined. The Canada Western Extra-Strong Red Spring (CWESRS) wheat class consists of only three

cultivars: Glenlea, Bluesky and Wildcat. These cultivars have extra-strong dough mixing properties required for this market class. Their HMW allelic constitution consisted of alleles *Glu-1A*$^{(b)}$, *Glu-1B*$^{(b)}$, *Glu-1D*$^{(d)}$ and the LMW allele *Glu-3D*$^{(c)}$(Lukow and Townley-Smith, 1996). This suggests that this allele could provide considerable improvement in the breadmaking potential for lines in Populations 1 and 2.

The *Glu-3A* and *Glu-3B* loci were characterized by the large differences among alleles and 1BL.1RS translocation, and the low frequency of the most favorable alleles, in both populations. A possible biochemical explanation for the large differences among *Glu-3* alleles is the close linkage between the LMW glutenin loci (*Glu-3*) with the gliadin loci *Gli-1* (i.e., *Gli-1A*, *Gli-1B* and *Gli-1D*), where each locus consists of several genes coding for γ- and ω-gliadins. Although the gliadins do not engage in polymer formation, these proteins have a plasticizer function that contribute to the physical properties of the dough (Wrigley, 1996). The proportions of γ- and ω-gliadins can vary considerably among cultivars according to Wieser *et al.*(1994). They found a weak negative correlation between SDS-sedimentation and the relative amount of ω-gliadins (r = -0.57). Their negative effect on quality is possibly due to the fact that these gliadins do not contain cysteine and consequently do not form disulphide bridges. Such bonds are important for their three-dimensional structure and function. These facts support the idea that the *Glu-3* alleles can be considered

as markers for complex genomic regions, with a large number of clustered genes coding for glutenins and gliadins (Sabelli and Shewry, 1991).

4.1.3- The Inter-locus Interactions

In Population 1 (Table 6), a significant interaction between HMW *Glu-1D* and LMW *Glu-3B* was found for dough strength. This demonstrates that the effects of these glutenin loci are not completely additive and the contribution of each allele varies according to the allele present at a different locus. A significant interaction between HMW *Glu-1D* and LMW *Glu-3A* was found in Population 2 for SDS and dough strength (Table 7), again confirming the importance of these loci and their differential response to allelic variation at other loci, e.g., the *Glu-1D* alleles contribution to dough quality varies according to the allelic composition at *Glu-3A*. It is important to note that the interactions did not change the relative ranking ($Glu\text{-}1D^{(d)} > Glu\text{-}1D^{(a)}$) in either population, for dough strength or SDS. The only exception was the interaction between *Glu-1D* and $Glu\text{-}3A^{(c)}$ allele, where allele $Glu\text{-}1D^{(a)}$ was significantly superior to allele $Glu\text{-}1D^{(d)}$ (data not shown).

4.1.4- The Hardness Contribution

For Population 1, the contribution of kernel hardness to the variation in SDS was significant (Table 6). In terms of tenacity, the interaction between the HMW *Glu-1D* locus and hardness was found to be significant (Table 6). This suggests that these factors are important in the determination of the viscoelastic properties of bread wheat. For Population 2, since 37 of the 40 lines were classified as hard, the contribution of hardness is not discussed.

The differences in LS means for SDS-sedimentation and dough strength were significant for both populations, with hard wheats being stronger than soft wheats (i.e., higher dough strength values)(Table 12 and 13). This is in agreement with the end product uses, where hard wheats are used by the breadmaking industry and soft wheats are used for pastry, cookies, etc.

The biochemical basis for the superiority of hard wheats over softs in terms of dough strength is unclear. A linkage has been suggested between one of the two genes that control the levels of free lipids in the grain and the major gene for hardness (*Ha*), as lipids are also important in the determination of viscoelastic properties of the dough. In addition, kernel hardness also affects starch damage, which is important in terms of water absorption and mixing properties.

Table 12- Least Square Means for SDS-Sedimentation Value and Alveograph W (Dough Strength) for the different classes of wheat in terms of hardness for Population 1(1995 and 1996).

	Hardness	
Class	SDS (ml)	W ($x10^{-4}$J)
Soft	$31.1^{b\dagger}$	155^{d}
Hard	37.5^{a}	194^{c}

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 13- Least Square Means for SDS-Sedimentation Value and Alveograph W (Dough Strength) for the different classes of wheat in terms of hardness for Population 2 (1995 and 1996).

	Hardness	
Class	SDS (ml)	W ($x10^{-4}$J)
Soft	$32.7^{b\dagger}$	163^{d}
Hard	37.5^{a}	218^{c}

†- LS Means in the same column followed by the same letter are not significantly different (P>0.05).

4.1.5- Summary

In summary, the results of this study point out the overwhelming importance of the glutenin allelic composition in the determination of wheat breadmaking quality. The methodology used to examine the contribution of the different LMW alleles at the three *Glu-3* loci appear to be correct, as the results for the HMW glutenins are in accordance with the literature. The conclusions drawn were reinforced by studying in parallel two different populations. The large differences in protein percentage between 1995 and 1996 also allowed for a broader scope in evaluating the role of the High and Low-Molecular-Weight subunits of glutenin.

The LMW allelic composition is important for any wheat breeding program involved with breadmaking quality. The differences in dough quality between the LMW alleles at *Glu-3A* and *Glu-3B* are substantial and the identification of alleles at these loci offer important directions in choosing parents for crossing. Selection of progeny in early generations can be also implemented, although with higher costs, as much larger numbers of lines would have to be examined. Another possibility is a backcrossing program, where the allelic composition at the six glutenin loci can be planned to give a high quality cultivar, with the necessary agronomic adaptation.

4.2- Recombinant Inbred Lines (Study "B")

4.2.1- The HMW Glutenin Contribution

This study consisted of two different populations of recombinant inbred lines (RILs) developed from the crosses BR-35 x ORL-9127 and ORL-9285 x ORL-92146. The segregation of five loci in the first population (B1) and four in the second (B2) were examined. The non-segregant locus was monomorphic *Glu-3D*, where all parents possess the allele *Glu-3D*$^{(a)}$, thus no segregation for this locus was expected. It was also possible to verify the results obtained for Populations 1 and 2 in the previous study. The advantage of this approach is that it allows for the measurement of the allelic effects on quality without the confounding effect of linkage disequilibrium. In the first study involving Populations 1 and 2, alleles that by chance are present in high quality lines may be incorrectly considered to be favorable to dough strength. With the generation of recombinant inbred line (RIL) populations, the different alleles are "randomized" in the population. Also, this approach allowed for comparisons in more uniform backgrounds, as the lines employed on each population are derived from a single cross.

The least square means for the *Glu-1A* locus confirms the ranking obtained from the previous study (Study "A"). It was found that alleles *Glu-1A*$^{(a)}$ and *Glu-1A*$^{(b)}$ were similar in their contribution to SDS-sedimentation for

Population B1 (Table 14), while in Population B2, where alleles *Glu-1A*$^{(b)}$ and *Glu-1A*$^{(c)}$ segregated, a significant difference was found, with allele *Glu-1A*$^{(b)}$ superior to the *Glu-1A*$^{(c)}$ allele (Table 15).

For *Glu-1B*, no differences were detected in SDS-sedimentation between the alleles in either populations, which agrees with the results in the first studies (Study "A").

For the *Glu-1D* locus, a difference in SDS-sedimentation between alleles *Glu-1D*$^{(d)}$ and *Glu-1D*$^{(a)}$ was found ($P < 0.0001$) in Population B1 (Table 14), confirming the results for Population 1 in the previous study. There was no segregation at this locus for Population B2, where both parents carried the favorable allele *Glu-1D*$^{(d)}$.

4.2.2- The LMW Glutenin Contribution

Significant differences between the alleles in SDS-sedimentation at the *Glu-3A* locus were found (Tables 14 and 15). For Population B1, the least square means confirms the findings from Study "A", that allele *Glu-3A*$^{(d)}$ was superior to allele *Glu-3A*$^{(c)}$. This superiority was also demonstrated in Population B2, where allele *Glu-3A*$^{(d)}$ was significantly superior to allele *Glu-3A*$^{(e)}$. Again, a

Table 14 - SDS-Sedimentation least square means for Experiment B1. The recombinant inbred lines (*n* =42) were classified according to their eletrophoretic pattern into one of the two possible allelic classes for each High and Low-Molecular-Weight glutenin locus.

Glu-1A		***Glu-1B***		***Glu-1D***		***Glu-3A***		***Glu-3B***	
Allele	SDS†	Allele	SDS	Allele	SDS	Allele	SDS	Allele	SDS
a	40.7[a‡]	b	39.4[b]	a	38.0[d]	*c*	38.8[f]	*f*	41.9[g]
b	39.2[a]	c	40.5[b]	d	41.9[c]	*d*	41.1[e]	1B.1R	38.0[h]

†- SDS-Sedimentation Values are expressed in milliliters (ml).
‡- Least Square Means in the same column followed by the same letter are not significantly different (P>0.05).

Table 15 - SDS-Sedimentation least square means for Experiment B2. The recombinant inbred lines (*n* =51) were classified according to their eletrophoretic pattern into one of the two possible allelic classes for each High and Low-Molecular-Weight glutenin locus.

Glu-1A		***Glu-1B***		***Glu-3A***		***Glu-3B***	
Allele	SDS†	Allele	SDS	Allele	SDS	Allele	SDS
b	47.3[a‡]	c	45.9[c]	*d*	46.7[d]	*f*	49.7[f]
c	43.2[b]	i	44.6[c]	*e*	43.8[e]	1B.1R	40.9[g]

†- SDS-Sedimentation Values are expressed in milliliters (ml).
‡- Least Square Means in the same column followed by the same letter are not significantly different (P>0.05).

null allele (*Glu-3A*$^{(e)}$) is expected to be inferior to most of the alleles and that was confirmed.

Both populations segregated for the presence of a wheat-rye translocation at the short arm of 1B chromosome. Therefore, it was not possible to compare *Glu-3B* alleles. However, it allowed for the comparison between a LMW *Glu-3B* allele with a 1BL.1RS chromosomal translocation (Tables 14 and 15). The superiority of the allele *Glu-3B*$^{(f)}$ over the 1BL.1RS translocation was confirmed in both populations.

4.3. The Wheat-Rye Chromosomal Translocation

Wheat-rye chromosome translocations have been employed in many wheat breeding programs in order to broaden adaptation, improve yield stability and to improve disease resistance (Rajaram *et al.*, 1983). However, these translocations are considered to confer inferior breadmaking properties to cultivars which carry such chromosome rearrangement (Dhaliwal *et al.*, 1990).

The results from Studies "A" and "B" confirm the negative contribution of the wheat-rye translocations to dough quality. For Study "A", alleles *Glu-3B*$^{(b)}$, *Glu-3B*$^{(d)}$, *Glu-3B*$^{(f)}$ and *Glu-3B*$^{(h)}$ were found to be superior to a 1B.1R wheat-rye translocation in terms of their contribution to quality (SDS-sedimentation and

dough strength) (Tables 12 and 13). For Study "B", allele *Glu-3B*$^{(f)}$ was found superior for SDS-sedimentation to the 1B.1R translocation as well (Tables 14 and 15).

These detrimental effects should be more clearly understood in order to better counteract them. The impact of translocations on SDS-sedimentation and Alveograph parameters should be made in uniform backgrounds. A third study (Study"C") was conducted with this purpose by employing three selected lines and their backcross derivatives. The cultivars BH-1146, Jupateco and Hartog were compared with translocated derivatives, including either chromosome 1AS or 1BS translocations, identified as experiments C1, C2 and C3, respectively.

The combined analysis of variance for Experiment C1 (Split-plot) indicates that years was an important factor influencing dough quality, although non significant (Table 16). This would be expected when protein percentage is not included in the analysis (covariate) and there are large differences between years for protein percentage. The factor Nitrogen (whole -plot) influenced significantly SDS-sedimentation, increasing SDS values with the application of additional nitrogen at heading. This is probably due to the increase in protein percentage induced by the treatment. The contribution of "Lines" (sub-plot factor) and "Years" were found to be significant for extensibility. The interaction "Lines x Years" was found significant for dough strength, indicating differences in the performance of lines between years. It is important to note that protein

Table 16- Means squares for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P) and Extensibility (L) for Experiment C1, for years 1995 and 1996. *Nitrogen* was the whole plot factor and *lines* were subplots.

Source	df	SDS	W	P	L
Lines	1	399	1800	2048	7503**
Nitrogen	1	116*	800	45	861
Line x Nitrogen	1	5	28	5	1
Nitrogen x Repl.(Year)	6	8*	94	7	178*
Replication(Year)	6	11	164	42*	114
Year	1	1140	15753	1596	18915*
Line x Year	1	53	6050*	72	1
Nitrogen x Year (Whole plot error)	1	53	450	1	78
Line x Year x Nitrogen	1	4	28	2	1
Error	12	2.1	62.0	9.9	60.6
C.V.%		4.0	4.6	3.9	9.6

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.

percentage was not included in the analysis as an explanatory variable, as the Nitrogen treatment directly influences this variable.

The combined analysis of variance for experiments C2 and C3 (Jupateco and Hartog) shows a strong interaction between Lines and Years for almost all measured traits(Tables 17 and 18). Consequently, the mean values for the traits under study are presented separately for 1995 and 1996. The significant effect of protein percentage on SDS-sedimentation and "Years" on extensibility was common for both experiments.

The comparisons between cultivars and their derivatives (Tables 19, 20 and 21) support the results from Studies "A" and "B" as the wheat-rye translocations have an unfavorable effect on the dough physical properties, reducing SDS-sedimentation, dough strength and extensibility, and increasing tenacity. The increase in tenacity with the decrease in extensibility causes a change in the ratio P/L, which measures the balance between the two components of dough strength. Therefore, the wheat-rye translocations have a tendency to produce tenacious doughs, contributing to mixing and handling problems. It is also evident from Tables 20 and 21 that the differences in SDS and dough strength between lines are larger at higher protein levels, reinforcing the conclusions drawn from the correlation coefficients between dough strength and protein percentages.

Table 17- Mean squares for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P) and Extensibility (L) for Experiment C2, for years 1995 and 1996.

Source	df	SDS	W	P	L
Lines	2	122.8	6801	183.4	222.6
Replications (Years)	6	1.6*	555	25.9	21.6
Years	1	0.1	143	243.8**	558.3*
Lines x Years	2	15.0***	4119***	212.0***	37.8
Protein %	1	7.0**	1091	38.9	7.7
Error	11	0.46	292	14.0	65.8
C.V.%		2.0	3.5	3.1	5.3

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.

Table 18- Mean squares for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P) and Extensibility (L) for Experiment C3, for years 1995 and 1996.

Source	df	SDS	W	P	L
Lines	2	71.2	1554	357.7	176.6
Replications (Years)	6	2.7*	224	12.0	9.5
Years	1	1.2	214	204.8**	182.2*
Lines x Years	2	17.7***	1974***	387.2***	361.6***
Protein %	1	14.6***	1967***	0.3	159.6*
Error	11	0.65	90.9	10.5	23.6
C.V.%		2.1	3.5	3.1	5.3

*,**,*** Significant at the 0.05, 0.01 and 0.001 probability levels.

Table 19- Means for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P), Extensibility (L) and Protein Percentage for Experiment C1, using two years of data (1995 and 1996).

	SDS (ml)	W ($x10^{-4}J$)	P (mm)	L (mm)	Protein %
BH-1146	$39.7^{a\dagger}$	177^{c}	71^{f}	97^{g}	13.2^{i}
BH-1146 *6/ Alondra‡	32.6^{b}	162^{d}	87^{e}	66^{h}	12.8^{j}
L.S.D.	1.1	6.1	2.4	6.0	0.18

†- Means in the same column followed by the same letter are not significantly different (P>0.05) based on the Least Significant Difference Test.
‡- BH-1146*6/ Alondra = BH-1146 1BL.1RS

Table 20- Means for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P), Extensibility (L) and Protein Percentage for Experiment C2, for the 1995 and 1996 years.

	SDS (ml)		W ($x10^{-4}J$)		P (mm)		L (mm)		Protein %	
Line	95	96	95	96	95	96	95	96	95	96
Jupateco	$45.8^{a\dagger}$	31.3^{d}	394^{a}	171^{c}	86^{a}	111^{c}	121^{a}	37^{b}	15.2^{a}	8.4^{b}
Jupateco*3/Amigo‡	36.3^{c}	27.3^{e}	300^{b}	168^{c}	89^{a}	129^{b}	107^{a}	31^{c}	15.5^{a}	8.7^{b}
Jupateco*6/Amigo	37.5^{b}	26.0^{e}	318^{b}	151^{d}	86^{a}	130^{b}	111^{a}	28^{c}	15.5^{a}	8.6^{b}
L.S.D.	0.64	1.28	43	11	6.5	6.1	18	3.7	0.7	1.0

†- Means in the same column followed by the same letter are not significantly different (P>0.05) based on the Least Significant Difference Test.

‡- Jupateco*3/ Amigo = Jupateco 1AL.1RS

Table 21- Means for SDS-Sedimentation Value, Alveograph Dough Strength (W), Tenacity (P), Extensibility (L) and Protein Percentage for Experiment C3, for the 1995 and 1996 years.

	SDS (ml)		W ($x10^{-4}J$)		P (mm)		L (mm)		Protein %	
Line	95	96	95	96	95	96	95	96	95	96
Hartog	$46.8^{a\dagger}$	34.0^{d}	346^{a}	209^{d}	79^{a}	115^{d}	143^{a}	47^{c}	14.3^{b}	8.8^{c}
Hartog*4/ Skorospelka-35‡	43.3^{b}	35.3^{d}	310^{b}	211^{d}	81^{a}	146^{b}	143^{a}	37^{d}	15.4^{a}	9.4^{c}
Hartog*4/ Amigo	38.5^{c}	30.5^{e}	311^{b}	235^{c}	80^{a}	126^{c}	126^{b}	51^{c}	15.3^{a}	9.8^{c}
L.S.D.	1.0	1.7	20	16	4.1	7.2	10.4	5.3	0.2	1.1

†- Means in the same column followed by the same letter are not significantly different (P>0.05) based on the Least Significant Difference Test.

‡- Hartog*4/ Skorospelka-35 = Hartog 1BL.1RS
Hartog*4/ Amigo = Hartog 1AL.1RS

A small effect of the wheat-rye translocations on protein percentage was observed (Tables 19 and 21), explaining the use of means instead of least square means (the covariant should not be affected by the treatments).

4.4. Rank of Genotypes

To compare the different genotypes from Populations 1 and 2 (Study "A") in terms of dough strength, the least square mean values for each line were calculated and the lines ranked within each population. To further examine the influence of the HMW and LMW glutenins on quality, the glutenin genotype for each line is presented in the lower chart. It was also calculated the single factor R-square value using the observations from the graph. This approach was used to explore the data from a different perspective.

In Figure 3 a bar chart demonstrates a wide range in dough strength, where each bar represents a line from Population 1. The contributions of each locus for individual genotypes (lower part) shows that *Glu-1D* is the most important locus controlling quality and explains more than half of the variation in dough strength. This reflects the high frequency of the 5+10 alleles in wheat germplasm in regions where historically breadmaking quality has been an objective for many decades. Contributions of the other five loci to dough strength are not large. However, it is possible to detect a clear pattern in the lower chart

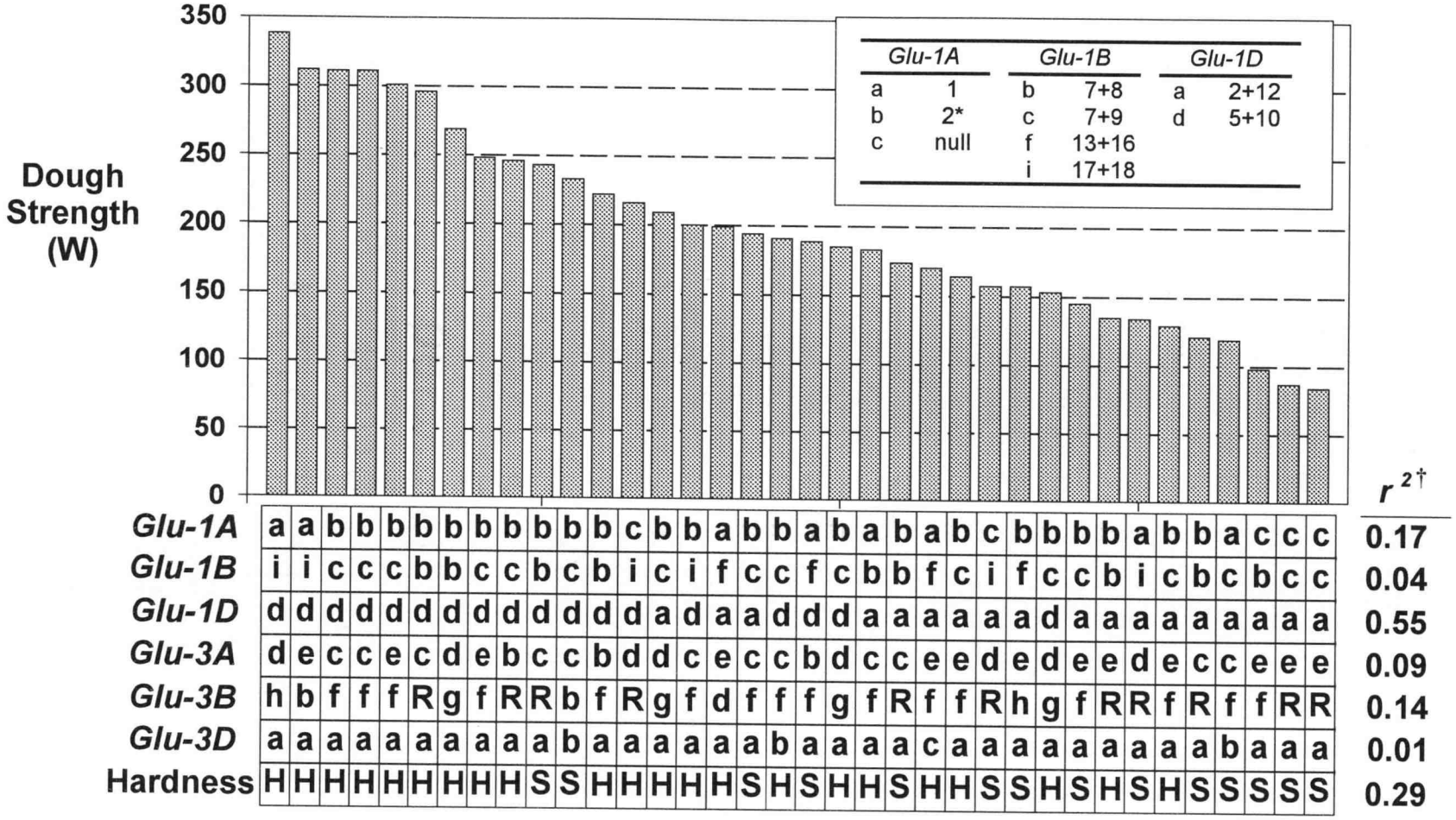

Figure 3- Dough strength for Population 1 wheat genotypes. Each bar is the least square mean of five replications in two years, using protein percentage as a covariate. The glutenin genotype of each line is presented in the lower chart, with hardness classification (H for hards and S for soft wheats). *Glu-3B* allele R stands for 1BL.1RS.
† - Refers to single factor R-square value using only the 36 observations in the graph.

with unfavorable alleles concentrated in the right side of the chart, indicating that quality is the overall result of the contributions of the seven factors studied. In other words, the poor performance of lines with low dough strength values is due to the accumulation of several unfavorable glutenin alleles and softness.

The second major factor was hardness, where hard wheats are usually stronger (higher dough strength) than softs. However, there is a large variation within hard and soft lines, as can be seen in Figure 3.

Population 2 had two important features in that most of the lines were hard wheats (37 of 40) and the allele *Glu-1D*$^{(d)}$ was present in 32 out of the 40 lines. The allelic constitution of Population 2 allowed for the study of factors that are most important when *Glu-1D* and hardness are constant. In this population the bar chart (Figure 4) reflects that this group of lines had a smaller range in dough strength compared to Population 1 (Figure 3). This was expected as allele *Glu-1D*$^{(d)}$ and hardness were constant. Even then, it possible to identify a trend towards the concentration of favorable alleles on the left side and unfavorable towards the right side to the chart.

The R-square values were calculated using a single locus in order to explain the variation in dough strength among the LS means for each line within each group (Figures 3 and 4). The R-square values indicate that LMW *Glu-3B*

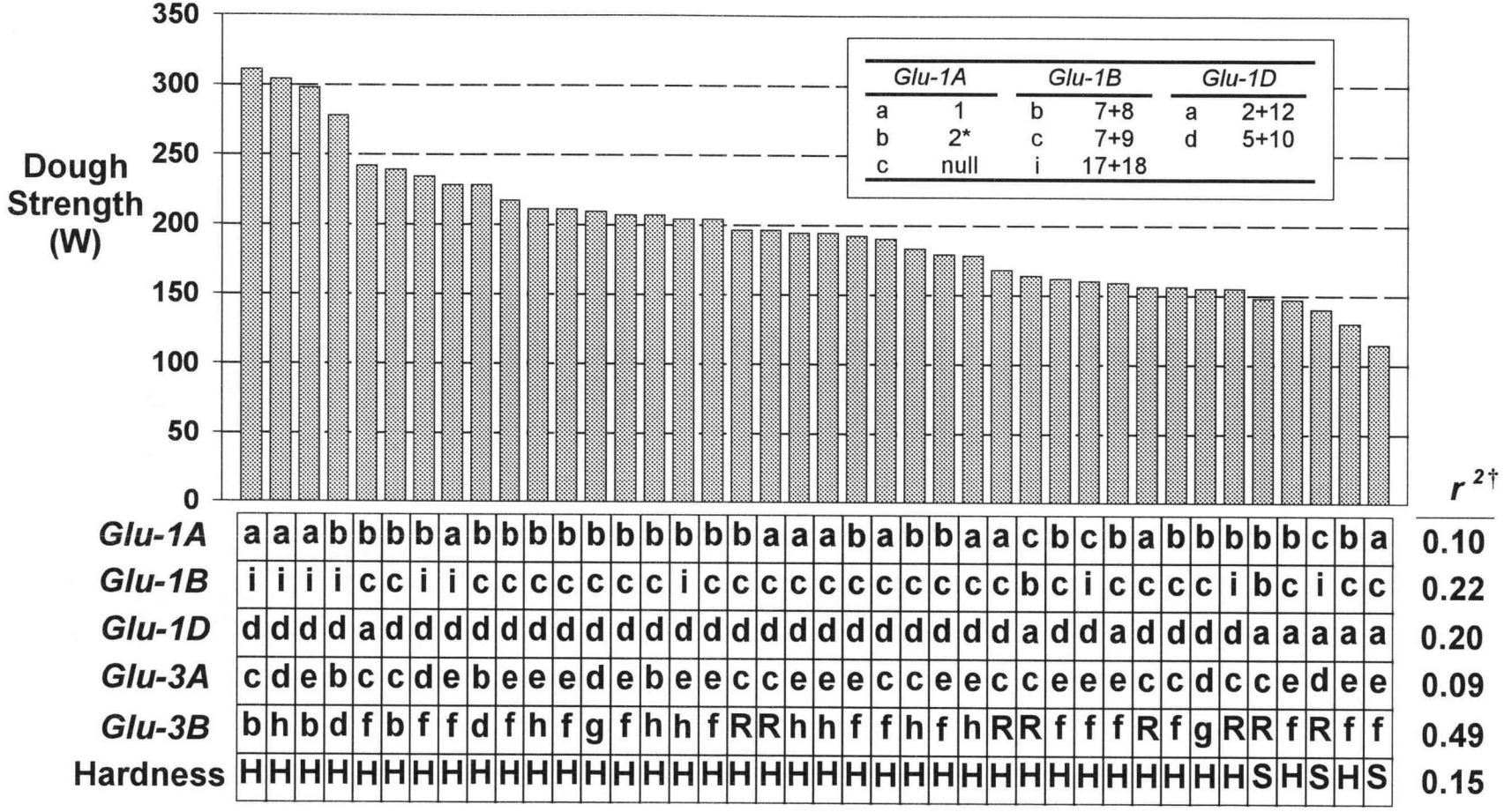

Figure 4- Dough strength for Population 2 wheat genotypes. Each bar is the least square mean of four replications in two years, using protein percentage as a covariate. The glutenin genotype of each line is presented in the lower chart, with hardness classification (H for hards and S for soft wheats). *Glu-3B* allele R stands for 1BL.1RS.
† - Refers to single factor R-square value using only the 40 observations in the graph.

was the most important locus controlling quality for Population 2, with a r^2 value of 0.49, whereas *Glu-1D* per se explained 20% of the variation in dough quality. It is important to understand that the relative importance of a locus depends on the allelic frequencies at all other loci: *Glu-3B* explained 14% of the variation for Population 1, where there are similar frequencies for the alternatives at *Glu-1D* (18 of 36 lines are *Glu-1D*$^{(d)}$) and hardness (22 of 36 lines are hard). *Glu-3A* explained the same amount of variation in dough quality in both populations (9%).

It is important to keep in mind the role of the inter-locus interactions in order to compare these results with the results from the previous analysis of variance (Tables 6 and 7), as the interactions were not considered in Figures 3 and 4. An important interaction was *Glu-1D* x *Glu-3B*, indicating that *Glu-3B* is an important locus and its relative contribution varies accordingly to the allelic composition at *Glu-1D*. It is important to stress that this interaction did not interfere with the rank among *Glu-3B* alleles. For Group 2, the importance of the *Glu-3B* locus is overwhelming in both analysis (Table 7 and Figure 2).

The graphical display of ranked lines simultaneously with the glutenin genotype and hardness attempted to provide wheat breeders with information about the relationship between dough strength and genotype and with an efficient method to identify the most favorable alleles for the trait of interest.

4.5. Implications on Breeding for Breadmaking Quality

The results demonstrate the contribution of the LMW glutenin allelic variation to breadmaking quality. A comparison between the contributions of the HMW and LMW will depend on the allelic frequencies at the three loci of each group. This was shown with HMW *Glu-1A*, where the allele null was infrequent in both groups (Appendix Tables 8 and 11), resulting in a non-significant overall effect for this locus (Tables 6 and 7). The contribution of *Glu-3A* and *Glu-3B* on the two populations was also evidence for this fact. In Population 1, where *Glu-1D* had both alleles at similar frequencies, the contribution of these LMW loci was not very expressive. On the other hand, in Population 2, where most of the lines were hard and carried allele *Glu-1D*$^{(d)}$, the contribution of *Glu-3A* and *Glu-3B* to SDS and W was substantial (Tables 6 and 7).

For the populations studied, it is clear that HMW *Glu-1D* is the most important glutenin locus controlling dough quality. The importance of other loci depends on the allelic frequencies in the population studied. For these populations, selection based on *Glu-1A* would be ineffective due to the high frequency of the most favorable alleles, i.e., *Glu-1A*$^{(a)}$ and *Glu-1A*$^{(b)}$. For *Glu-1B*, where the most detrimental alleles *Glu-1B*$^{(a)}$, *Glu-1B*$^{(d)}$ and *Glu-1B*$^{(e)}$(Payne *et al.*, 1984) are not present in these populations, selection against these alleles also would be ineffective.

In contrast, *Glu-3A* and *Glu-3B* offer a large potential for improvement in these populations, with large differences among alleles and a low frequency of the most favorable alleles (allele *Glu-3A*$^{(d)}$ and alleles *Glu-3B*$^{(b)}$ *Glu-3B*$^{(d)}$ and *Glu-3B*$^{(h)}$). *Glu-3D* exhibited the least polymorphism, where allele *Glu-3D*$^{(a)}$ was found in high frequency. The comparison with the three cultivars considered to be extra-strong in Canada suggests that other alleles (i.e., allele *Glu-3D*$^{(c)}$) can provide considerable improvement for these populations in terms of breadmaking potential. The introduction of such alleles into the germplasm groups is substantially more difficult without the determination of the LMW electrophoretic patterns.

Increments in the breadmaking potential can be important when it is considered the negative correlation between yield and protein content (Lorenzo and Kronstad, 1987, Carrilo *et al.* 1990). The generation of higher yields promotes lower protein contents, that cause reductions in dough strength. Improvement on the glutenin allelic composition, with the selection of the most favorable alleles at the six glutenin loci, can compensate for lower protein contents and preserve breadmaking quality potential.

The presence of wheat-rye chromosomal translocations is beneficial for yield potential, broad adaptation and yield stability (Rajaram *et al.* 1983). Their

negative effects on quality were evident from Studies "A", "B" and "C". The selection of the most favorable alleles for quality at glutenin loci not involved in the translocation (both HMW and LMW) is a potential strategy to compensate for the detrimental effects of these translocations and still benefit from their agronomic advantages.

From a breeder's point of view, the characterization of the LMW glutenin alleles in terms of their contribution to dough quality can also be important when a "parent building" program is undertaken to improve breadmaking quality. Faster progress should be feasible using backcrosses and selecting at one or two loci at a time. In a second phase, selected lines can be intercrossed in order to bring together the most favorable glutenin alleles into the adapted high-yielding cultivars. Another possibility for a breeding program is to test the newly formed advanced lines for quality and determine their glutenin allelic composition, developing a database that could become extremely useful after a few years. This might provide information on the effect on quality of new alleles being introgressed into the germplasm. The statistical analysis for this dataset should account for the differences in genetic background of the new lines each year (most of the lines would have just one or two years of data, as new lines are created and most of the tested lines are eliminated).

The identification of the most favorable glutenin alleles can provide important information for laboratories involved with wheat transformation. Blechl

and Anderson (1996) expressed hybrid HMW glutenin in transgenic wheat demonstrating that it is possible to create new alleles and thus increase the amount of glutenin subunits relative to other seed proteins. In order to move in this direction it is extremely important to understand the contribution of the HMW and LMW glutenins to the dough physical properties of wheat.

5. Summary and Conclusions

Three experiments were designed to study the contribution of the High and Low-Molecular-Weight glutenins (HMW and LMW) to breadmaking quality in wheat. Besides the six glutenin loci and their allelic variation, the effect of kernel hardness and the quantitative effect of protein were also evaluated. The variation in protein percentage between years provided a comprehensive understanding about the relationships between HMW and LMW allelic variation and quality under a wide range of protein percentage.

The basic strategy was the use of different approaches to investigate each issue related to dough quality. For Study "A", the use of two groups of lines allowed for the study of the contributions of the different loci at different allelic frequencies and provided information about the effect of selection for the most favorable HMW alleles. In Study "B" two populations of recombinant F_6 inbred lines from different crosses were tested for quality and their allelic composition determined. This allowed for the measurement of the allelic effects on dough quality without the confounding effect of linkage disequilibrium. Study "C" was established to investigate the contribution of the wheat-rye translocations to quality using three cultivars and near-isogenic lines for the wheat-rye translocations. This provided the opportunity to evaluate the effect of the translocations in uniform backgrounds involving different protein percentage levels. The conclusions are appropriate for the populations selected and provide

a broad understanding about the relationships between wheat's HMW and LMW glutenin allelic variation with the dough viscoelastic properties. Based on the results of these studies the following conclusions were made:

1. Breadmaking quality evaluation of wheat genotypes should be done at the higher protein levels (above 12%), where larger differences due to the genetic composition exist. At lower protein levels, responsive lines can be clustered with unresponsive lines, hindering a satisfactory evaluation.

2. The SDS-Sedimentation Test correlates significantly with Alveograph Dough Strength (r = 0.77 to r = 0.88) indicating that this inexpensive test can be useful to estimate dough strength and, therefore, might be used in early generation (F_3)selection in a breeding program.

3. *Glu-1D* is the most important locus controlling breadmaking quality. The comparison between alleles shows that allele *Glu-1D*$^{(d)}$ is superior to *Glu-1D*$^{(a)}$ for dough strength. Selection for allele *Glu-1D*$^{(d)}$ can be carried out using half kernels for SDS-PAGE electrophoresis and the embryos carrying the favorable alleles advanced. Subsequent progeny can also be selected using the same approach.

4. The comparison between the contribution of the HMW and LMW glutenins depends on the allelic frequencies at the three loci of each group. The

relative contribution of the LMW glutenins to the variation in dough quality is higher when the most favorable alleles at the HMW glutenins are constant (i.e., alleles *Glu-1A*$^{(a)}$ or *Glu-1A*$^{(b)}$, alleles *Glu-1B*$^{(b)}$,*Glu-1B*$^{(c)}$ or *Glu-1B*$^{(i)}$, and allele *Glu-1D*$^{(d)}$).

5. The 1BL.1RS wheat-rye chromosomal translocations have a detrimental effect on the dough physical properties of wheat. They cause a reduction in SDS-sedimentation volume, Alveograph dough strength and extensibility, with an increase in tenacity. A possible strategy to counteract these negative effects is the improvement of the allelic composition at the remaining glutenin loci to compensate for the absence of a glutenin locus, while benefiting from the agronomic advantages several wheat-rye translocations provide.

6. Inter-locus interactions were detected and explained part of the variation in dough quality. The interactions generally did not cause rank order changes, which simplified the rank of alleles at each locus. Interactions were also detected between the glutenin loci and hardness.

7. The lack of polymorphisms at *Glu-3D* in Populations 1 and 2 (Study "A") indicates that the existing variability at this locus is not being explored. In order to reverse this situation, it is necessary to identify lines with other alleles. It is also known that there are other alleles at *Glu-3A* and *Glu-3B*, which also should be introduced into these germplasm groups.

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Appendix

Appendix Table 1- Population 1 cultivars and advanced lines, with crosses and pedigrees.

	Genotype	Cross	Pedigree
1.	BR-23		
2.	BR-35		
3.	CEP-24		
4.	BR-18		
5.	Klasic		Privately developed cultivar
6.	OR-Juanito	CAR 853/ COC// Vee's'/3/ Bow's'	
7.	OR-1	PF-869107's'/ Bau's'	CM 104628 0M-20U-51Y-1U-0U
8.	PF-869107		
9.	ORL-91256	BR-23/ PF-869107's'	N 384-6U-1U-1U-1U-0U
10.	ORL-92171	PF-869107's'/ Bau's'	CM 104628-0M-18U-0U-1U-5U-1U-0U
11.	ORL-9322	PF-869107/ M1029-89	N 935-1U-1U-2U-0U
12.	ORL-9324	PF-87512/ KL Cartucho	N 944-4U-4U-2U-0U
13.	ORL-9325	CMH 794.955/3*CNO79// Coker-762/ BR-14// SA-815	N 670-2U-1U-2U-0U
14.	ORL-9356	PF-869107//PF-869155/ CEP14	N 593-A-2U-6U-5U-0U
15.	ORL- 9388	PF-869107/ Milan// PF-869107	N 1756-A-3U-3U-1U-0U
16.	ORL-93118	PF-869107/ KL H3450 c3111// BR-38	N 1747-B-1U-6U-1U-0U
17.	ORL-93131	PF-843025/ Don Ernesto// PF-869107	N 1778-A-2U-6U-2U-1U-0U
18.	ORL-93135	BCN/ PF-869107"s"// BR-35/3/ PF-87167	N 1808-C-1U-2U-1U-1U-0U
19.	ORL-93144	CNT7/ 6/ Bau"s"/7/ PF-843025/ BR-35	N 1339-E-2U-3U-2U-2U-0U
20.	ORL-93157	BR-35//BR-35/ Vee#5	N 1610-1U-2U-2U-1U-0U
21.	ORL-93177	PF-869107/ Rabe//BR-35	N 1753-D-2U-1U-1U-1U-0U
22.	ORL-93208	PF-869107/ CEP-14/BR-18	N 590-A-2U-1U-4U-1U-0U
23.	ORL-93220	CMH 794.955/3*CNO79//BR-35/3/ OC-16/ PF-869107	N-1348-B-1U-1U-2U-1U-0U
24.	ORL-93225	PF-869107// PF-869107"s"/ Kauz"s"	N 604-2U-1U-1U-4U-0U
25.	ORL-93234	CEP-85128/3/.../4/ ORL-91256	N 1338-5U-4U-1U-3U-0U
26.	ORL-93244	CNT7/... /6/ Bau"s"/7/ PF-869107"s"/Bau"s"	N 1155-1U-4U-1U-1U-0U
27.	ORL-93264	PF-87915/ KL H3394 s3110	N 1027-2U-1U-1U-4U-0U
28.	ORL-93301	PF-869107/ KL H3450 c3111	N 933-1U-1U-2U-4U-1U-0U
29.	ORL-93320	PF-869107/ KL H3450 c3111	N 933-1U-1U-1U-1U-2U-0U
30.	ORL-93321	PF-869107/ KL H3450 c3111	N 933-1U-1U-1U-1U-3U-0U
31.	ORL-9133	BR-23/ PF-869107's'	N 384-15U-2U-3U-0U
32.	ORL-91331	BR-23/ PF-869107's'	N 384-15U-5U-5U-2U-0U
33.	ORL-9285	PF-869107/ KLEIN H 3450 c3111	N 933-1U-1U-1U-3U-0U
34.	ORL-91274	PF-87512/ BR-15	N 388-2U-0U-1U-1U-0U
35.	ORL-92168	PF-85373/ Kauz's'	CM 104628-0M-18U-0U-1U-5U-1U-0U
36.	ORL-92146	SPRW/5/... /6/Vee#5/7/2*BR-23	CMBW89Y00893-0TOPM-0Y-2U-4U-2U-0U

Appendix Table 2- Population 2 cultivars and advanced lines, with crosses and pedigrees.

	Genotype	Cross	Pedigree
1.	BR-23		
2.	BR-35		
3.	BR-18		
4.	KLASIC	Privately developed cultivar	
5.	ORL-93582	PF-869107"s"/Bau"s"/4/PF70354/... /3/Bau"s"	N1157-3K-4K-1K-0U
6.	ORL-93595	OC-17*2 / PF-87451	N1464-1K-1K-3K-0U
7.	ORL-93613	PF-869107 // IOC-866 / PF-869107	N1171-1K-3K-2K-0U
8.	ORL-93615		N1171-1K-4K-3K-0U
9.	ORL-93624	IOC-866 / 2*PF-869107	N1198-2K-3K-1K-0U
10.	ORL-93639	Bacanora//PF-869107/ Bau"s"	N1096-3K-2K-5K-0U
11.	ORL-93649	PF-84316 / IAPAR-Caete	N746-2K-3K-1K-0U
12.	ORL-93664	OC-16/PF-869107// OC-16	N1705-A-7K-3K-2K-0U
13.	ORL-93667		N1705-A-7K-4K-1K-0U
14.	ORL-93668		N1705-A-7K-5K-1K-0U
15.	ORL-93673		N1705-A-8K-1K-1K-0U
16.	ORL-93679	PF-869107 / OC-16	N1533-4K-3K-2K-0U
17.	ORL-93686	PF-869107 / Kauz"s"	N1534-7K-2K-1K-0U
18.	ORL-93702	Bau"s"/BR-18 / PF-8722	N1627-8K-5K-2K-0U
19.	ORL-93709	BR-35"s"#2 / 2*BR-36	N1669-3K-4K-3K-0U
20.	ORL-93713	IOC-866 / PF-869107 // Gen	N1691-7K-1K-3K-0U
21.	ORL-93714		N1691-7K-1K-4K-0U
22.	ORL-93728	PF-70354/ Ald"s"//.../6/ PF-87227	N1154-1K-1K-1U-0U
23.	ORL-93731		N1154-6K-1K-2U-0U
24.	ORL-93733	BR-18*2 / PF-8722	N1402-2K-1K-1U-0U
25.	ORL-93739	OC-17*2 / PF-87451	N1464-3K-1K-1U-0U
26.	ORL-93743	PF-869107"s"/ Bau"s"// IOC-866 / BR-18	N1472-1K-1K-1U-0U
27.	ORL-93744		N1472-1K-1K-4U-0U
28.	ORL-93746	Coker 762/ BR-18 // OC-16	N709-3K-1K-1U-0U
29.	ORL-93748	PF-869107 // IOC-866 / PF-869107	N1171-1K-3K-2U-0U
30.	ORL-93753		N1171-1K-4K-4U-0U
31.	ORL-93754	IA-8429 / Milan"s"// BR-23	N1193-1K-2K-3U-0U
32.	ORL-93758	IOC-866 / 2*PF-869107	N1198-1K-2K-2U-0U
33.	ORL-93761		N1198-2K-2K-3U-0U
34.	ORL-93762		N1198-2K-2K-4U-0U
35.	ORL-93786	Vee#5"s"/ PF-869107	N769-1K-2K-2U-0U
36.	ORL-93791	OC-16 / PF-869107 // OC-16	N1705-A-5K-2K-5U-0U
37.	ORL-93793		N1705-A-7K-3K-5U-0U
38.	ORL-93796	PF-869107 / BR-26	N1531-3K-1K-2U-0U
39.	ORL-93798	PF-869107 / OC-16	N1533-2K-2K-1U-0U
40.	ORL-93804	IOC-866 / PF-869107 // Gen	N1691-3K-1K-1U-0U

Appendix Table 3- Population 1 list with their HMW and LMW subunit allelic composition, with hardness classification and combination number.

Line	*Glu-1A*	*Glu-1B*	*Glu-1D*	*Glu-3A*	*Glu-3B*	*Glu-3D*	*Hardn.*	*Comb.*
1.	c	i	a	d	R	a	S	1
2.	b	b	a	c	R	a	S	2
3.	b	c	d	c	b	b	S	3
4.	a	i	d	e	b	a	H	4
5.	a	i	d	d	h	a	H	5
6.	b	c	d	b	R	a	H	6
7.	b	c	a	d	g	a	H	7
8.	b	c	a	e	f	a	S	8
9.	a	i	a	d	R	a	S	9
10.	b	c	d	d	g	a	H	10
11.	a	f	a	e	d	a	H	11
12.	a	f	a	e	f	c	H	12
13.	b	b	a	c	R	a	S	2
14.	b	f	a	e	h	a	S	13
15.	b	c	a	e	f	a	H	14
16.	b	b	d	b	f	a	H	15
17.	b	c	a	c	f	a	S	16
18.	a	b	a	c	f	a	H	17
19.	b	b	a	e	R	a	H	18
20.	c	b	a	e	f	a	S	19
21.	b	b	d	c	R	a	S	20
22.	a	f	d	b	f	a	S	21
23.	b	i	d	c	f	a	H	22
24.	a	c	a	c	f	b	S	23
25.	b	c	d	c	f	b	H	24
26.	b	c	d	d	g	a	H	10
27.	b	b	d	c	R	a	H	25
28.	b	c	d	e	f	a	H	26
29.	b	c	d	c	f	a	H	27
30.	b	c	d	c	f	a	H	27
31.	c	c	a	e	R	a	S	28
32.	c	c	a	e	R	a	S	28
33.	b	c	d	e	f	a	H	26
34.	b	c	a	e	f	a	H	14
35.	b	b	d	d	g	a	H	29
36.	c	i	d	d	R	a	H	30

Appendix Table 4- HMW and LMW glutenin subunit alleles found in Population 1, with hardness classification. Numbers within parenthesis refer to old nomenclature.

Glu-1A	*Glu-1B*	*Glu-1D*	*Glu-3A*	*Glu-3B*	*Glu-3D*	*Hardn.*
a (1)	b (7+8)	a (2+12)	b	b	a	S (soft)
b (2*)	c (7+9)	d (5+10)	c	d	b	H (hard)
c (null)	f (13+16)		d	f	c	
	h (17+18)		e	g		
				h		
				R†		

†- Refer to the presence of a wheat-rye 1BL.1RS translocation.

Appendix Table 5- Population 2 list of lines with their HMW and LMW subunit allelic composition, with hardness classification and combination number.

Line	*Glu-1A*	*Glu-1B*	*Glu-1D*	*Glu-3A*	*Glu-3B*	*Hardn.*	*Comb.*
1.	null	17+18	2+12	d	R	Soft	1
2.	2*	7+8	2+12	c	R	Soft	2
3.	1	17+18	5+10	e	b	Hard	3
4.	1	17+18	5+10	d	h	Hard	4
5.	2*	7+9	5+10	e	f	Hard	5
6.	null	7+8	2+12	c	R	Hard	6
7.	2*	7+9	5+10	e	f	Hard	5
8.	1	7+9	5+10	c	R	Hard	7
9.	2*	7+9	5+10	e	f	Hard	5
10.	2*	7+9	5+10	c	R	Hard	8
11.	2*	17+18	5+10	b	d	Hard	9
12.	1	7+9	5+10	e	h	Hard	10
13.	2*	7+9	5+10	e	h	Hard	11
14.	1	7+9	5+10	e	h	Hard	10
15.	2*	7+9	5+10	c	h	Hard	12
16.	2*	7+9	5+10	b	d	Hard	13
17.	2*	7+9	2+12	c	f	Hard	14
18.	2*	7+9	5+10	c	b	Hard	15
19.	1	7+9	5+10	c	R	Hard	16
20.	1	7+9	5+10	c	f	Hard	17
21.	1	7+9	5+10	c	R	Hard	16
22.	null	17+18	5+10	e	f	Hard	18
23.	2*	17+18	5+10	d	f	Hard	19
24.	1	17+18	5+10	c	b	Hard	20
25.	2*	17+18	5+10	c	R	Hard	21
26.	2*	7+9	5+10	d	g	Hard	22
27.	2*	7+9	5+10	d	g	Hard	22
28.	2*	17+18	5+10	e	h	Hard	23
29.	2*	7+9	2+12	e	f	Hard	7
30.	2*	7+9	2+12	e	f	Hard	7
31.	2*	7+9	2+12	e	f	Hard	7
32.	2*	7+9	5+10	e	f	Hard	5
33.	2*	7+9	5+10	e	f	Hard	5
34.	2*	7+9	5+10	e	f	Hard	5
35.	1	7+9	2+12	e	f	Soft	24
36.	1	17+18	5+10	e	f	Hard	25
37.	1	7+9	5+10	e	h	Hard	10
38.	2*	7+9	5+10	e	f	Hard	5
39.	2*	7+9	5+10	b	h	Hard	11
40.	2*	7+9	5+10	c	f	Hard	26

Appendix Table 6- HMW and LMW glutenin subunit alleles found in Population 2, with hardness classification. Numbers within parenthesis refer to old nomenclature.

Glu-1A	*Glu-1B*	*Glu-1D*	*Glu-3A*	*Glu-3B*	*Glu-3D*	*Hardn.*
a (1)	b (7+8)	a (2+12)	b	b	a	S (soft)
b (2*)	c (7+9)	d (5+10)	c	d		H (hard)
c (null)	h (17+18)		d	f		
			e	g		
				h		
				R†		

†- Refer to the presence of a wheat-rye 1BL.1RS translocation.

Appendix Table 7- Study "C" lines and backcross derivatives:

Experiment	Lines	Genotype	Origin
C1	BH-1146	normal	Brazil
	BH-1146 * 6/ Alondra	1BL.1RS	Brazil
C2	Jupateco	normal	Mexico
	Jupateco * 3/ Amigo	1AL.1RS	Brazil
	Jupateco * 6/ Amigo	1AL.1RS	Brazil
C3	Hartog†	normal	Australia
	Hartog*4/ Skorospelka-35	1BL.1RS	Australia
	Hartog*4/ Amigo	1AL.1RS	Australia

†- Gupta and Sheperd (1992)

Appendix Table 8- Classification of Population 1 lines into allelic groups for the three High-Molecular-Weight glutenin loci (*n* =36).

Glu-1A		*Glu-1B*		*Glu-1D*	
Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
a	8	b	9	a	18
b	23	c	17	d	18
c	5	f	4		
		i	6		

Appendix Table 9- Classification of Population 1 lines into allelic groups for the three Low-Molecular-Weight glutenin loci (*n* =36).

Glu-3A		*Glu-3B*		*Glu-3D*	
Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
b	3	b	2	a	32
c	12	d	1	b	2
d	8	f	16	c	1
e	13	g	4		
		h	2		
		R	11		

Appendix Table 10- Classification of Population 1 lines into two hardness groups (*n*=36).

Hardness	
Type	Frequency
Soft	14
Hard	22

Appendix Table 11- Classification of Population 2 lines into allelic groups for the three High-Molecular-Weight glutenin loci (*n* =40).

Glu-1A		*Glu-1B*		*Glu-1D*	
Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
a	12	b	2	a	8
b	25	c	28	d	32
c	3	i	10		

Appendix Table 12- Classification of Population 2 lines into allelic groups for the three Low-Molecular-Weight glutenin loci (*n* =40).

Glu-3A		*Glu-3B*		*Glu-3D*	
Alleles	Frequency	Alleles	Frequency	Alleles	Frequency
b	3	b	3	a	40
c	13	d	2		
d	5	f	17		
e	19	g	2		
		h	8		
		R	8		

Appendix Table 13- Classification of Population 2 lines into hardness groups (*n*=40)

Hardness	
Type	Frequency
Soft	3
Hard	37

Appendix Table 14- Summary of meteorological data for Corvallis, Oregon (1995).

	Average Temperature, °C			Precipitation,
Month	Max.	Min.	Mean	mm
January	9.6	3.6	6.6	251.5
February	12.6	3.5	8.0	109.0
March	13.6	3.0	8.3	120.4
April	15.1	3.9	9.5	134.6
May	20.9	7.5	14.2	36.3
June	22.8	10.0	16.4	59.9
July	27.8	12.8	20.3	13.2
August	26.4	10.0	18.2	20.8

Appendix Table 15- Summary of meteorological data for Corvallis, Oregon (1996).

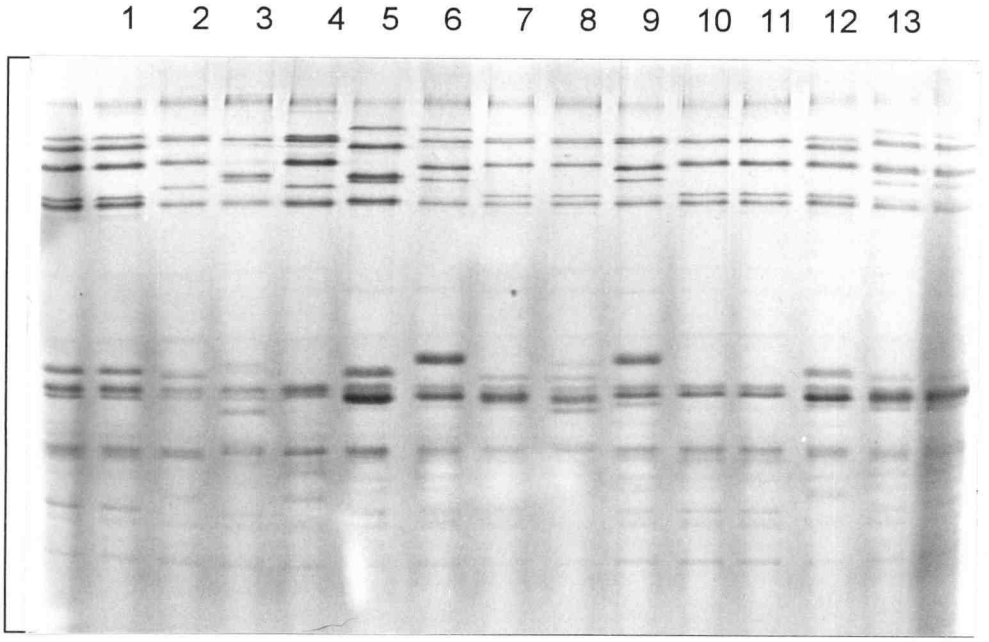
	Average Temperature, °C			Precipitation,
Month	Max.	Min.	Mean	mm
January	8.6	2.8	5.7	263.4
February	9.9	1.3	5.6	346.6
March	13.7	3.5	8.6	89.9
April	16.5	5.8	11.2	125.2
May	17.5	6.2	11.8	101.1
June	23.0	8.4	15.7	21.6
July	29.7	12.3	21.0	23.4
August	28.6	11.3	20.0	3.6

Appendix Table 16- Mean Squares for SDS-sedimentation for Population B1 (Study "B") in 1996.

Source	df	SDS
Combinations	25	33.5*
Glu-1A	1	7.9
Glu-1B	1	0.6
Glu-1D	1	96.8*
Glu-3A	1	26.2
Glu-3B	1	73.6*
Hardness	1	7.1
Glu-1A x Glu-1D	1	46.2
Glu-1A x Glu-3A	1	23.8
Glu-1A x Glu-1B	1	7.0
Glu-1A x Glu-3B	1	5.8
Glu-1B x Glu-1D	1	13.8
Glu-1B x Glu-3A	1	9.7
Glu-1B x Glu-3B	1	5.5
Glu-1B x Hardness	1	7.7
Glu-1D x Glu-3A	1	3.8
Glu-1D x Hardness	1	3.8
Glu-3A x Hardness	1	2.1
Glu-1A x Glu-1B x Glu-1D	1	23.2
Glu-1A x Glu-1D x Glu-3A	1	10.3
Glu-1B x Glu-1D x Glu-3A	1	3.7
Glu-1B x Glu-1D x Hardness	1	0.7
Glu-1B x Glu-3A x Glu-3B	2	7.7
Glu-1B x Glu-3A x Hardness	1	7.4
Glu-1D x Glu-3A x Hardness	1	10.3
Lines (Combinations)	16	15.5***
Replications	1	117.6***
Protein	1	282.8***
Error	40	3.3
CV%		4.8

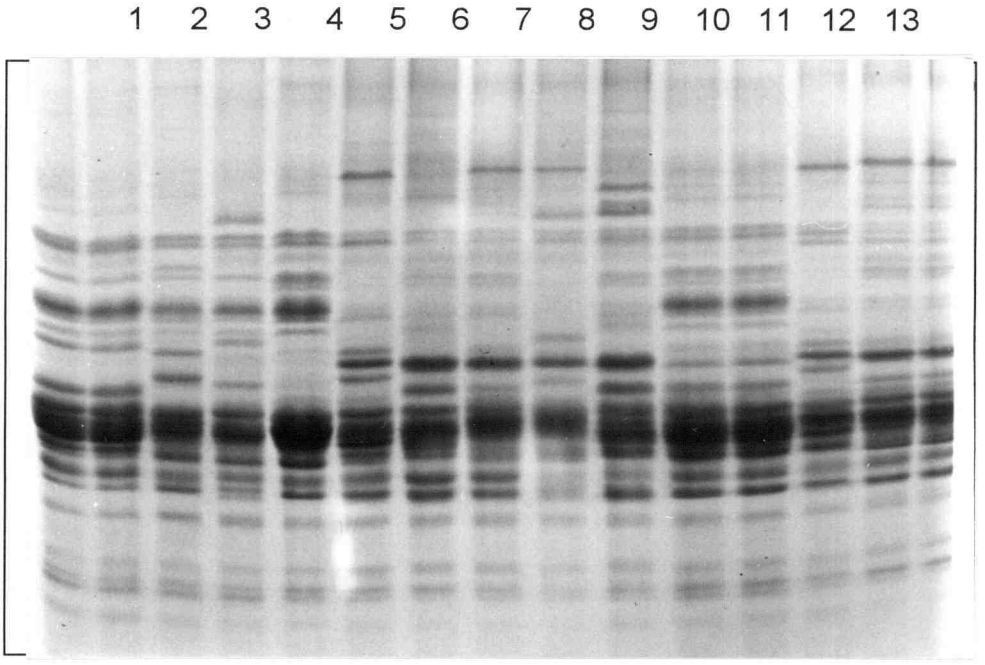
Appendix Table 17- Mean squares for SDS-sedimentation for Population B2 (Study "B") in 1996.

Source	df	SDS
Combination	10	234.0***
Glu-1A	1	263.6**
Glu-1B	1	15.5
Glu-3A	1	89.1
Glu-3B	1	394.5***
Glu-1A x Glu-1B	1	13.7
Glu-1A x Glu-3A	1	20.6
Glu-1A x Glu-3B	1	0.0
Glu-1B x Glu-3A	1	0.9
Glu-3A x Glu-3B	1	61.6
Glu-1A x Glu-1B x Glu-3A	1	17.6
Lines (Combinations)	40	32.3***
Replications	1	14.0**
Protein	1	426.2***
Error	49	1.9
CV%		2.9



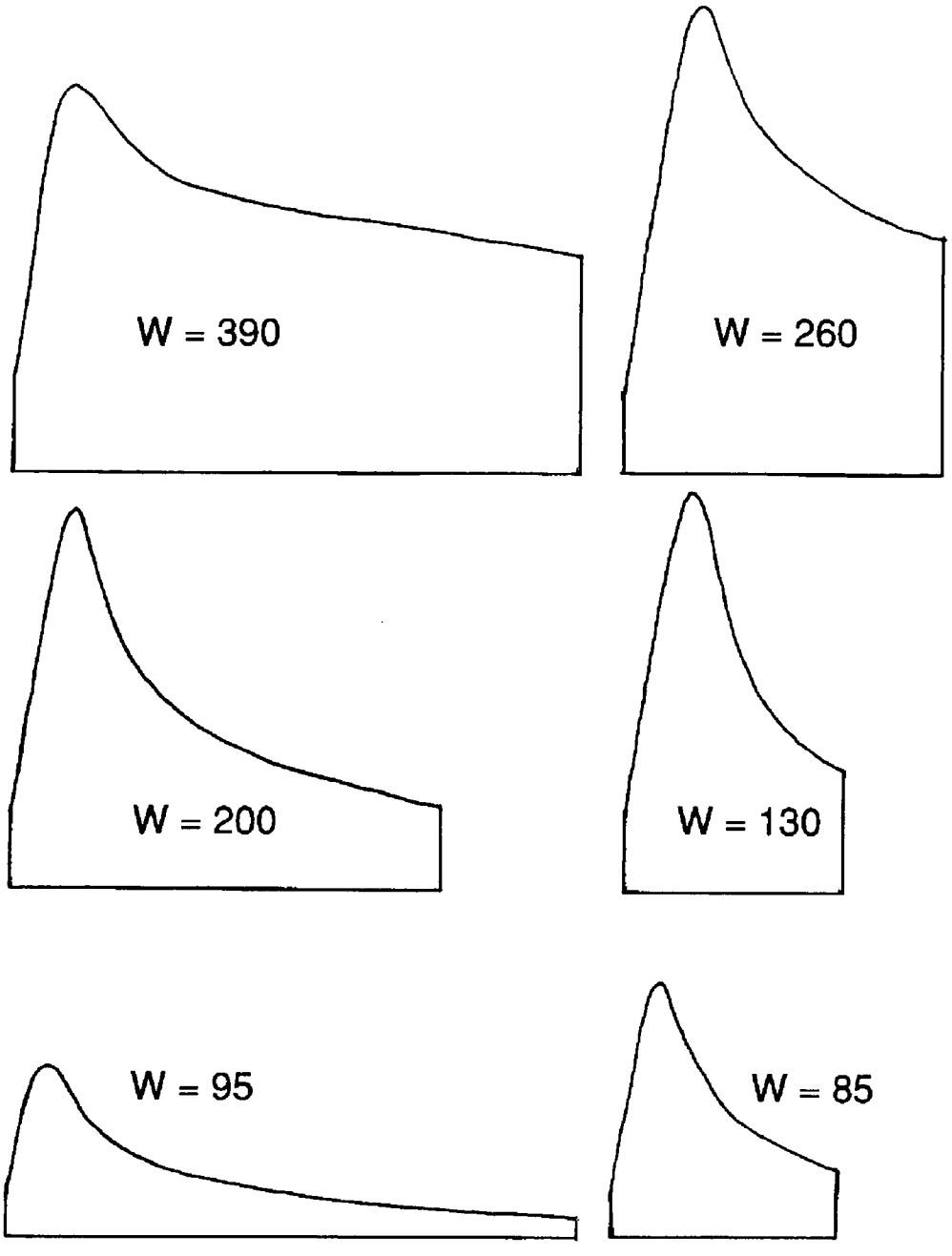
Appendix Figure 1- SDS-PAGE banding patterns of the High-Molecular-Weight (HMW) and Low-Molecular-Weight (LMW) glutenins for a series of lines suggested as standards for identification of LMW alleles.

Lane	*Glu-1A*	*Glu-1B*	*Glu-1D*	*Glu-3A*	*Glu-3B*	*Glu-3D*
1	2*	7+9	5+10	**b**	R	a
2	2*	7+8	2+12	**c**	R	a
3	null	17+18	2+12	**d**	R	a
4	2*	7+8	2+12	**e**	R	a
5	1	17+18	5+10	c	**b**	a
6	1	13+16	2+12	e	**d**	a
7	2*	7+9	2+12	e	**f**	a
8	2*	7+9	2+12	d	**g**	a
9	2*	13+16	2+12	e	**h**	a
10	null	7+9	2+12	e	**R**	a
11	null	7+9	2+12	e	R	**a**
12	2*	7+9	5+10	c	b	**b**
13	1	13+16	2+12	e	f	**c**



Appendix Figure 2- SDS-PAGE banding patterns of unreduced gliadins for a series of lines suggested as standards for identification of LMW Glu-3B alleles and wheat-rye translocations (1BL.1RS).

Lane	*Glu-3A*	***Glu-3B***	*Glu-3D*
1	b	**R**	a
2	c	**R**	a
3	d	**R**	a
4	e	**R**	a
5	c	***b***	a
6	e	***d***	a
7	e	***f***	a
8	d	***g***	a
9	e	***h***	a
10	e	***R***	a
11	e	**R**	a
12	c	**b**	b
13	e	**f**	c



Appendix Figure 3- Alveographs for samples of cultivars Klasic, BR-35 and ORL-9133 (top to bottom), illustrating large differences among cultivars in dough strength (W). Alveographs on the left side are from high protein samples, while alveographs on the right are from low protein samples.